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PHYSIOLOGICAL PRE-DETERMINATION: THE INFLUENCE OF THE PHYSIOLOGICAL CONDITION OF THE SEED UPON THE COURSE OF SUBSEQUENT GROWTH AND UPON THE YIELD.

V. REVIEW OF LITERATURE. CHAPTER IV.

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(With Plate I.)

CHAPTER IV

THE EFFECT OF CONDITIONS DURING GERMINATION AND IN THE EARLY SEEDLING STAGE UPON SUBSEQUENT GROWTH AND FINAL YIELD.

INTRODUCTION.

IN the previous chapter of this review we (18) dealt with seed-treatments which could be classed as treatments affecting nutrition. The predetermining effect of increased or decreased nutritional supply operating for a limited period during the critical stages of early development was demonstrated. The proportionality established between seedling weights due to differential nutrition is maintained when the nutritional supply

is subsequently equalised during the main period of growth, and it is directly reflected in the final yield.

In the light of evidence afforded by growth curves based on dry-weight measurements which were available in the literature, growth and development within the limits of hereditary potentialities were treated as fundamentally matters of physiological pre-determination. The general expression covering growth was described as the Compound Interest Law of Development. The operation of this law may be subject to a number of secondary modifications.

A few fundamental principles are necessary for the study of growth and development. These are conspicuous by their absence in existing text-books of plant physiology, which excel in the assemblage of interesting curiosities and of uncorrelated details. The phenomena of *normal growth* seem to call for further study and analysis and for the application of mathematical treatment. Even a clear grasp of the general conception of the compound interest law of development at once greatly simplifies the handling of problems of physiological pre-determination and of growth. One is able to formulate approximately the magnitude of effects that will be observed as the result of causes operating continuously or for a short time in relation to the stage of development at which the cause operates and the effect is desired.

In the cases with which we dealt in the preceding chapter the absolute magnitude of the final effects upon yield were out of all proportion to the absolute magnitude of the causes to which they were due. These causes operated for a short period during the early stages of development and initiated what we have termed physiological pre-determination. It is this fact which makes the phenomenon of physiological pre-determination of such importance from the economic point of view. Roughly speaking, the absolute value of the effect produced is proportional, not to the value of the initial cause (*i.e.* that which has to be supplied by the agriculturist), but to the value of the initial cause multiplied by the time-interval between the operation of the cause and the reaping of the result.

In the present chapter we deal with seed-treatments which we have classified for convenience under the following headings, viz.:

(1) Physical treatments of the seed;

(2) Chemical treatments of the seed, which do not obviously affect its nutrition[1].

[1] It should be pointed out that there are many papers which deal with the effect upon *germination* of various chemical treatments of seeds, but in which no mention is made of the pre-determining effects of the seed-treatments (cf. Bokorny (4); Sigmund (32)).

PHYSICAL TREATMENTS OF THE SEED.

(*a*) HIGH TEMPERATURES.

Experimental work in which seeds in the air-dry condition have been submitted to high temperatures falls into three categories.

It has been established that in the case of many seeds the capacity for germination, though low after the harvest, increases during storage to its full value, which is attained by the following spring. A typical example of the results obtained by Atterberg (2) in the case of barley is given below (Table I).

TABLE I.

Barley reaped at four stages.

Stage of maturity of the seed when harvested at the end of September	Percentage of Moisture in the seed: When reaped at the end of September	Percentage of Moisture in the seed: On the 20th of October, after having been stored in a cold barn and then taken to the laboratory	Percentage of germination when sown on: Sept. 26th	Oct. 5th	Oct. 20th	Oct. 27th	Nov. 3rd	Nov. 19th	Dec. 2nd
Green	42	19·0	21	6	1	29	45	82	95
Green	39	18·6	17	3	2	31	36	85	94
Yellow ripe	35	18·3	17	2	0	21	30	90	98
Ripe	27	18·4	11	1	4	3	21	85	98

It has been shown by numerous workers (*e.g.* Hotter (16); Velten (36); Atterberg (2), and others) that if the seeds are exposed for a short time to relatively high temperatures during the period of low germination they at once attain their full capacity for germination (cf. Table II).

TABLE II (after Atterberg).

Kind of seed	Treatment of the seed	Result of germination test
Barley	Dried for 2 days at 37° C.	Gave 22 % germination in 4 days
	,, 4 ,, ,,	,, 50 % ,, ,, 12 ,,
	,, 6 ,, ,,	,, 98 % ,, ,, 10 ,,
	,, 8 ,, ,,	,, 99·5 % ,, ,, 8 ,,
	The control seed (untreated) gave only 4 % germination	

We have here a definite experimental result, but a full explanation of the underlying causes of the change which occurs during storage and which is accelerated by heat is as yet not forthcoming, neither, so far as we have been able to find, are there any records extant as to the influence, if any, this procedure exercises upon the development and final yield of the plants produced.

In the second category we have a large amount of experimental work which has been undertaken with the object of obtaining a simple method of sterilising seeds. In this work tests of the growth and yield of the plants from treated seeds as compared with that of the controls from untreated seeds have been carried out, but in considering the yields obtained the authors have not distinguished between the specific physiological effects of the heat-treatment and the effect of seed sterilisation.

Finally we have a category into which fall experiments carried out with the definite object of ascertaining the pre-determining effect upon growth and yield of exposing seeds to relatively high temperatures (cf. Sprengel (33); Ockel (25); Pietrusky (27); Krašan (21); Velten (36); and Wollny (43 & 44)). The results obtained are contradictory, and no definite conclusion can be drawn from them.

The most striking of the earlier results appear to have been obtained with flax by Pietrusky. A brief exposure of the air-dry seed to temperatures ranging from 30° to 50° C. increased the quality and yield of the fibre produced by the experimental plants.

Wollny (43 & 44) in the case of a number of cereals and other economic crops reached the conclusion that dry heating the seed generally increased the productivity of the plants produced. Some of the results obtained by Wollny are summarised in the following tables (Tables III and IV):

TABLE III (after Wollny).

Experiments carried out in 1876.

Kind of seed	Number of seeds	Weight of the experimental seeds on April 5th	Weight of the experimental seeds on April 30th	Percentage loss in weight during the drying-process	Weight of the untreated seeds	Temperature maintained during the drying-process
White lupins	200	82·49 gm.	80·07 gm.	2·93	83·30 gm.	30°–35° C.
Peas	200	68·23	64·75	5·10	67·80	30°–35° C.
Flax	—	100	96·95	3·05	99·30	30°–35° C.

Harvest Results.

Kind of seed	Condition of seed sown	Percentage of plants at the harvest	Yield from 100 plants: Seeds	Yield from 100 plants: Straw
Lupin	Dried at 30°–35° C.	87	371 gm.	406 gm.
	Untreated	95	264	347
Peas	Dried at 30°–35° C.	75	1381	1594
	Untreated	91	1143	1410

			Yield per 4 sq. m.	
			Seeds	Fibre
Flax	Dried at 30°–35° C.	—	290 gm.	1595 gm.
	Untreated	—	222	1208

The area allowed for each plant was 25 sq. cm. in the case of the lupins and peas. The flax seed was sown broadcast over an area of 4 sq. metres. All seed was sown on May 4th.

TABLE IV (after Wollny).

Experiments carried out in 1876–77.

Kind of seed	Number of seeds	Weight of the experimental seeds: Before drying	After drying	Percentage loss in weight during the drying-process	Weight of the untreated seeds	Temperature maintained during the drying-process
Winter rye	100	4·79 gm.	4·49 gm.	6·25	4·79 gm.	30°–35° C.
Maize	75	36·40	34·93	4·04	36·40	30°–35° C.
Peas	100	40·77	38·44	5·71	41·61	30°–35° C.
Lupin	100	44·51	42·25	5·08	45·08	30°–35° C.
Flax	—	80·00	76·39	4·52	79·85	30°–35° C.

Harvest Results.

Kind of seed	Condition of the seed sown	Percentage of plants at the harvest	Number of haulms per 100 plants	Yield from 100 plants: Seed	Straw
Winter rye	Dried at 30°–35° C.	82	693	1091 gm.	2378 gm.
	Untreated	85	497	808	1412
Peas	Dried at 30°–35° C.	50	—	1638	3125
	Untreated	99	—	1522	2714
Lupin	Dried at 30°–35° C.	83	—	1153	3172
	Untreated	91	—	1519	4304

			Yield from 100 plants: Green	Air-dry
Maize	Dried at 30°–35° C.	38	111,250 gm.	74,166 gm.
	Untreated	47	94,000	63,000

		Yield per 4 sq. metres: Seeds	Total weight	Chaff	Weight of 500 of the harvested seeds
Flax	Dried at 30°–35° C.	283 gm.	955 gm.	238 gm.	2·20 gm.
	Untreated	341	832	236	2·27

The area allowed for each plant was 20 sq. cm. in the case of the rye, and 25 sq. cm. in the case of the maize, peas, and lupins. The flax was sown broadcast. The seed was sown on May 4th, 1877, with the exception of the winter rye, which was sown on September 22nd, 1876.

An analysis of the effect of this treatment of the seed upon the development of the plant was carried out subsequently in considerable detail by Wollny (44), who showed that (1) the percentage of germination was decreased in some cases (*e.g.* peas, beans, rye), in others (*e.g.* wheat,

barley, oats) not appreciably; (2) the growth of the resulting plant was at first delayed; and (3) the experimental plants flowered earlier and more freely than the controls. He also found that seeds after dry heating were unusually sensitive to climatic and soil conditions during germination. As a result of this growth-analysis Wollny appears to have been doubtful as to the utility from the economic point of view of drying seeds.

(*b*) Low Temperatures.

Many authors have dealt with the effect upon germination of exposing seeds in the moist condition to low temperatures. This has been found to be a method of general application for stimulating the germination of dormant seeds (cf. Kinzel (20)).

The question as to what effect exposure to low temperatures during the critical stages of germination may have upon the subsequent course of development has been investigated in the case of seeds of the so-called winter-cereals which normally germinate in late autumn, pass the winter in the vegetative condition, and flower during the following summer. If the seeds are sown in the spring, the plants produced do not complete their development during the ensuing summer and autumn but they flower and set seed only in the following summer.

This behaviour on the part of winter-annuals has attracted attention in relation to problems of rhythm and periodicity, the question at issue being whether rhythm and periodicity in plants are inherent properties of the protoplasm or the direct effects of external conditions. Without entering into a discussion of the theories put forward by various authors we may deal fully with the careful series of experiments recently carried out by Gassner (10)[1] at Hamburg, and published last year. Gassner planted several winter varieties of cereals[2] in the spring and investigated whether exposure of the seeds during germination or of the plants during the early stages of seedling growth to low temperatures would affect their subsequent development. In the only communication at present available this author confines his attention to the time of formation of the flowering stems (culms) of the cereals investigated.

In each experiment four lots of 50 seeds ("pure line") were germinated on moist sand in crystallising dishes, which were kept at tempera-

[1] Gassner gives a full list of references to previous work on this subject.

[2] For example, "Petkuser" winter rye; "Friedrichswerther Mammuth" winter barley; "Svalöfs Extra Squarehead" winter wheat, etc.

tures of 1°–2° C., 5°–6° C., 12° C., and 24° C. respectively. When germination was complete (*i.e.* when the cotyledon had attained a length of from 2 to 2·5 cms.) the seedlings were rinsed with water to remove sand, and the 20 most vigorous were forthwith planted out in four large flower-pots filled with good compost. After one day, during which they were kept in the shade, these flower-pots were sunk in the ground in a part of the Botanic Gardens where uniform illumination was assured, and from the time of germination onwards the plants were grown under similar external conditions.

Curves *A* and *B* in Fig. 1 give the temperatures obtaining at 1 p.m. and at 7 a.m. respectively from March 1st to September 30th. The time of flower-stem formation was reckoned from the day on which at least one flower-spike of a plant in a series had emerged at least half-way from its surrounding leaf-sheath.

His results may be briefly summarised as follows. Exposure of the seeds to low temperatures during germination or during the early seedling stage ensures that flowering shall occur during the first year (Plate I, Fig. I; and cf. Appel and Gassner (1)). He carried the analysis further and obtained the following quantitative results. Firstly, the lower the temperature (over a range of 0°–25° C.) the shorter the interval between germination and culm-formation as is shown in Fig. 2, which we have constructed from Gassner's data. Secondly, the earlier in the development of the plant the exposure to the low temperature occurs, the more marked the result (see Tables V and VI; and cf. Gassner (9)).

TABLE V.

Effect of a low temperature at different stages in the development of "Petkuser" winter rye.

Date of sowing	Treatment following the sowing		Date on which the seedlings were planted out*	Result
March 18th	A. Kept at 24° C. for 30 hours	Followed by a temperature of 1°–2° C. for 4 weeks	April 16th	Formed flowering-stems irregularly from the beginning of August
	B. Kept at 1°–2° C. for 4 weeks	Followed by a temperature of 24° C. for 30 hours		Formed flowering-stems regularly from June 25th onwards

* On April 16th the cotyledons of the plants belonging to series A were about 20 mm. in length whereas those belonging to series B were about 30 mm. in length.

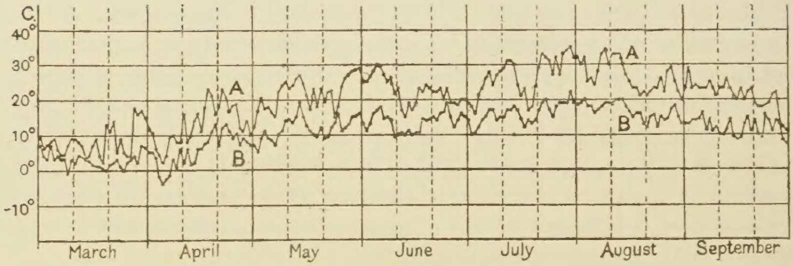

C.
40°
30°
20°
10°
0°
-10°
A
B
A
B
March
April
May
June
July
August
September

Fig. 1.

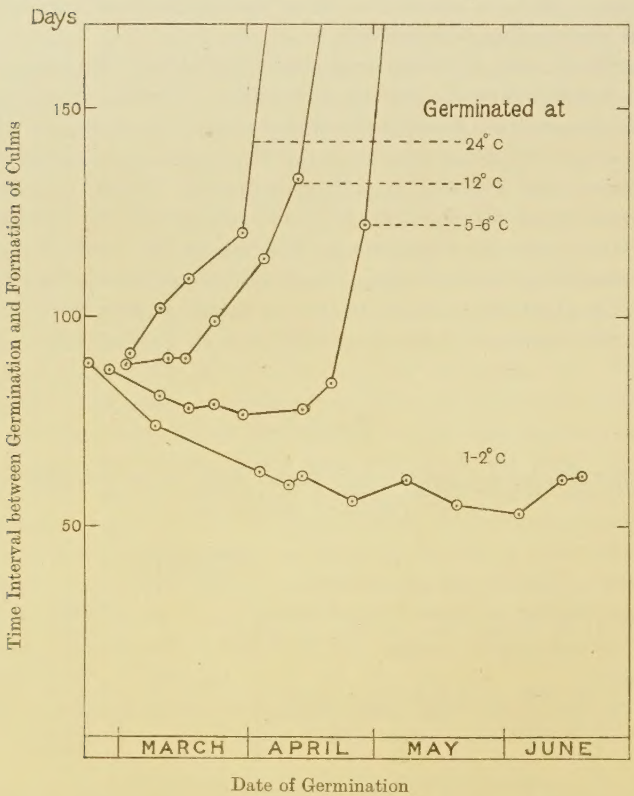

Days
150
100
50
Time Interval between Germination and Formation of Culms
Germinated at
24° C
12° C
5-6° C
1-2° C
MARCH
APRIL
MAY
JUNE
Date of Germination

Fig. 2.

TABLE VI.

Effect of a low temperature at different stages in the development of "Uruguay" oats.

	Treatment	Result of treatment
Seeds kept at 6°–9° C. for 5 days	Followed by a temperature of 25° C. until germination was completed	Plants flowered during the same season
Seeds kept at 25° C. for 1–2 days	Followed by a temperature of 6°–9° C. for 5–7 days (*i.e.* until germination was completed)	Plants did not flower during the same season

If the exposure to the low temperature occurs 2–3 days after sowing at normal temperatures (*e.g.* 24° C.) it is necessary that the period of exposure to the low temperature be many times as long in order to obtain the same result as when the exposure is made during the first few hours after sowing (see Table VII).

TABLE VII.

Influence of low temperature on the time of culm-formation in "Petkuser" winter rye sown in the spring.

Treatment: Germination period at 24° C.	Treatment: Followed by a period at 0° to −5° C.*	Result of the treatment
3 days	25 days	Formed flowering-stems the same year after 68 days
3 "	3 "	Did not form flowering-stems the same year
3 "	1 "	Did not form flowering-stems the same year
3 "	0 "	Did not form flowering-stems the same year

* The plants were subsequently grown under natural conditions.

In parallel experiments carried out with so-called summer-annuals of the same varieties it was found that exposure to low temperatures during germination did not appreciably influence the time at which culm-formation took place[1] (see Fig. 3).

(*c*) ELECTRICAL DISCHARGE.

Results of empirical experiments dealing with the effects of electrical discharge upon seeds during germination are on record. The interesting aspect of these experiments from our point of view lies in the fact that it is stated that brief exposure of seeds prior to and during germination

[1] Gassner and Grimme (11) have shown that plants which require a period of low temperature to bring about complete development, can withstand extreme cold, whereas plants which do not need a low temperature, are unable to withstand cold.

influences not only the vigour and percentage of germination, but also the whole course of subsequent growth and final yield. It is interesting in this connection to note the fact that from the records of experiments in which growing crops have been submitted to electrical discharge throughout their development there is reason to believe that beneficial results are most obvious during the initial stages of growth.

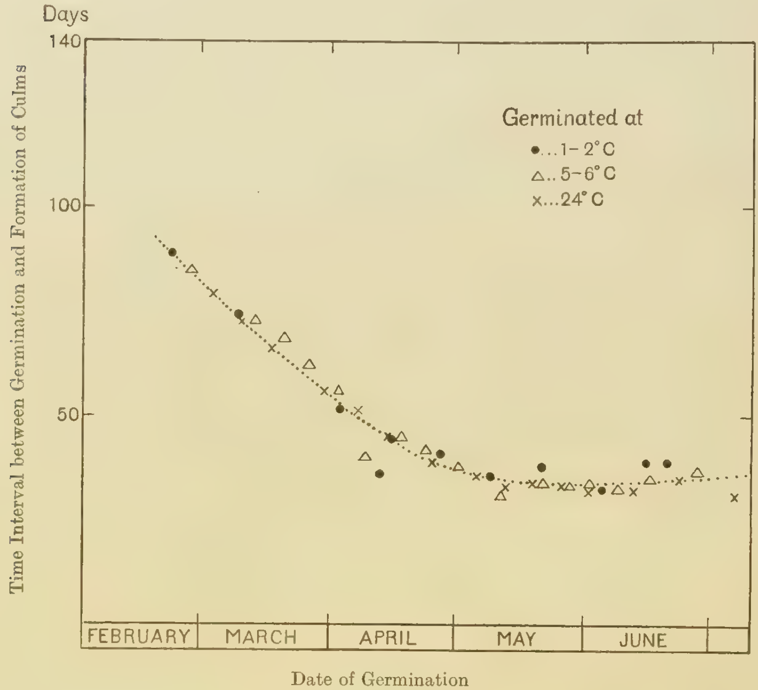


Fig. 3.

The following table (Table VIII) summarises certain recent results obtained at the Research Station for Plant Physiology at Dahlem, which were reported by Höstermann (15) in the *Landwirthschaftliche Jahrbücher* for 1913. The main significance of these results, apart from the fact that effects were found to last throughout the life of the plant, seems to be that better results are obtained when the seeds are treated after swelling in water than when treated dry, and that injury can easily result from too powerful a discharge.

TABLE VIII.

A. *Experiments with Seeds as the Positive Electrode.*

Kind of seed used and its condition during experiment	Distance of discharging electrode from the seeds	Length of spark	Duration of discharge	Effect of treatments upon germination and subsequent growth
Dry seeds of *Phleum pratense* sown in germination dishes	5 cm.	22 mm.	1 min.	Has a *favourable* influence upon germination and also upon the subsequent growth
		32 mm.		Has an *unfavourable* influence upon the later growth
Swollen seeds of *Phleum pratense* sown in germination dishes	5 cm.	12 mm.	1 min.	The germination power is *greatly decreased* probably because the seeds were swollen for too long a period and because the discharge was too intense
Dry seed of SPRING RYE sown in germination dishes	5 cm.	12 mm.	1 min.	No effect
			2 mins. 3 ,, 5 ,,	These treatments have an initial *favourable* action upon germination and produce a *final small increase in growth*
			10 ,,	Causes injury and delay in growth

B. *Experiments with Seeds as the Negative Electrode.*

Kind of seed used and its condition during experiment	Distance of discharging electrode from the seeds	Length of spark	Duration of discharge	Effect of treatments upon germination and subsequent growth
Dry seeds of SPRING RYE sown in germination dishes	40–50 cm.	40 mm.	½ hour 1 ,, 1½ hours 2 ,,	Result in an *increase of germination* and of *ear-formation*, but a *decrease in growth*
Swollen seeds of BARLEY; these seeds had been previously soaked in water for 6 hours at 30° C. and were afterwards sown in the open	5 cm.	32 mm.	2 mins. 10 ,, 30 ,,	The effect of these treatments is at first to increase growth considerably, but the difference disappears later
Swollen seeds of SPRING WHEAT; these seeds had been previously soaked in water for 6 hours at 30° C. and were afterwards sown in the open	5 cm.	23 mm.	2 mins. 10 ,, 30 ,,	The effect of these treatments is to increase growth; this *increase in growth is maintained.* Ear-formation was doubled by the 10-minute treatment

Swollen seeds of SPRING WHEAT; these seeds had been previously soaked in water for 6 hours at 30° C. and were afterwards sown in the open	5 cm.	32 mm.	2 mins. 10 ,, 30 ,,	*Increased growth was obtained* particularly after the 2 minute and 10 minute periods * * This experiment was repeated with similar results
Swollen seeds of SPRING RYE; previously soaked in water at 30° C. for: 1 hour 3 hours 5 ,, 6 ,, 7 ,, 8 ,,	10 cm.	32 mm.	15 mins.	The best results upon subsequent growth were obtained with the seeds which had been soaked in water for 6, 7, and 8 hours respectively
Swollen seeds of SPRING RYE; soaked in water at 30° C. for 72 hours and then sown in the soil	40 cm.	40 mm.	30 mins.	Resulted in an *increase in germinating power and in growth energy*
Swollen seeds of SPRING WHEAT; previously soaked for 5 minutes Placed in germination dishes	2 cm. ,, ,, ,, ,,	27 mm. ,, ,, ,, ,,	2 mins. 5 ,, 10 ,, 20 ,, 30 ,,	No acceleration in germination (2, 5, 10 mins.) *Considerable delay in germination* (20, 30 mins.)
Swollen seeds of SPRING WHEAT; previously soaked for 5 minutes Placed in germination dishes	2 cm. ,, ,, ,, ,, ,,	12 mm. 22 ,, 27 ,, 32 ,, 40 ,, 50 ,,	10 mins. ,, ,, ,, ,, ,,	Results: small acceleration at lower voltages; slight injury at moderate voltages; decided injury at high tensions (*i.e.* with spark lengths of 40 mm. and 50 mm.)

According to Micheels and De Heen (23) the action of a high frequency alternating current upon wheat and pea seeds, which had previously been soaked in water for 24 hours, favourably affected the development of the seedlings, but unfortunately their observations extended over a few days only.

(*d*) X-RAYS.

Increased vigour of germination and increased growth of the resulting plant, so far as it has been followed, has been recorded by several authors as the result of brief treatment of the seeds to X-rays. An example may be quoted. Promsy and Drevon (31) found that exposure of swollen seeds for ½–1 hour to the influence of X-rays markedly increased the subsequent growth of the seedlings. This action of the X-rays occurred only when the seeds were kept at a relatively high temperature (40° C. *circa*) during the exposure. For details with regard to the rays used and the conditions of exposure, which were carefully recorded by the investigators, reference should be made to the original paper. The following table (Table IX) has been based upon the results obtained by Promsy and Drevon:

TABLE IX.

Experiment I.

White lupin seeds were soaked in water for 15 hours, after which the testas were removed. On the first 3 days the experimental seeds were exposed to the X-rays for 1 hour, ½ hour, and ½ hour respectively, the temperature being 40–45° C. On the 4th day the seeds were sown in damp sand together with the controls (untreated).

Results.

Results on	Treated*	Untreated
4th day	Average length of radicles = 16·7 mm.	Average length of radicles = 6·2 mm.
18th „	Shoots appeared	—
26th „	—	Shoots appeared
29th „	Average height of plants above level of sand = 9·9 cm.	Average height of plants above level of sand = 7·3 cm.

* The treatment also brought about certain anatomical modifications.

Experiment II.

Seeds of haricot beans were soaked in water for 2 hours after which they were exposed to X-rays for 1 hour (temp. 40° C.).

Results.

	Treated	Untreated
Average length of plants on the 15th day	10 cm.	5 cm.
Average dimensions of		
1. Cells of the cortex	55·0 μ	44·0 μ
2. Cells of the pith	48·0 μ	43·0 μ
3. Nuclei of the cortical cells ...	18·0 μ	11·1 μ
4. Nuclei of the pith cells	15·0 μ	13·2 μ

CHEMICAL TREATMENTS OF THE SEED.

(*a*) ACIDS.

A large number of workers (*e.g.* Fischer (8), Onodera (26), and others) have noted the stimulating effect of acids upon germination. We have only been able to find a few cases in which observation was extended to the subsequent course of development of the treated seeds, but whenever this has been done, it is interesting to find that the author has usually recorded favourable results of a more or less striking nature. A few examples may be quoted.

Promsy (30), having first established the fact that the effect upon germination of a continuously-acting acid medium was favourable in a

large number of trials, employed the method of soaking the seeds *for a short time* in the acid solutions and then sowing them in garden soil.

Seeds of a variety of *Cucurbita Pepo* were soaked for 48 hours in the following solutions, viz.:

0·5 % Tartaric acid	(*T*)
0·5 % Acetic acid	(*A*)
0·5 % Oxalic acid	(*O*)
Pure water	(*W*)

After this preliminary soaking treatment the seeds were washed in water and were then sown in sand in perforated dishes. *A* came up first, *T* next, followed by *O*, and several days later by *W*. Measurements taken sometime later (*i.e.* when the seedlings bore from 2–6 leaves) showed that the *A* plants had the largest number of leaves and also the greatest dry-weight, whereas the *W* plants had the smallest number of leaves and the smallest dry-weight. Forty days after sowing the perforated dishes were half buried in garden soil, but a single plant from each lot was transplanted. The final results in the case of those not transplanted were as follows. All the *W* plants died; of the others, flowers appeared first upon the *A* plants, and these plants also bore the largest and most numerous fruits. All the plants from the acid-treated seeds maintained their vigour throughout the experiment.

Of the transplanted plants, *A* produced three very large fruits, *O* produced two, *T* produced one, whilst *W* flowered, but did not fruit. Results of a similar nature were obtained by this author with Pepper seeds.

The main interest of these results lies in the fact that a brief treatment during the critical stage of germination was found to produce a beneficial effect on the whole course of subsequent development of the plants and also upon their final yields.

We may recall here the incidental observations of three other workers, namely, Goodspeed, Townsend, and Plate. Goodspeed (12), when testing the effect of 80 per cent. H_2SO_4 upon the germination of tobacco seeds, found that treatment with this acid for a period not exceeding 10 to 12 minutes markedly increased the percentage of germination, and in some cases the rapidity of germination also. This result is in harmony with the results of many other investigators on the effect of strong acids upon the germination of hard-coated seeds. From our point of view, however, Goodspeed's most interesting contribution lies in a sentence hidden in the body of his paper—"Results at present at hand seem to leave no

doubt that the action of sulphuric acid is further strikingly effective in increasing the rate of growth during at least the first three months of the plant's life." This observation of Goodspeed's is supported by results recorded by F. Plate (29). Working with seeds of *Avena sativa* Plate noticed that after treatment with various inorganic acids the germination of the seeds was greatly accelerated, and that the plants produced from the treated seeds were more vigorous than the controls. He observed that the reserve materials were exhausted in the case of the acid-treated seeds in 10 days as against 15 days in the case of untreated seeds.

The effect of hydrocyanic acid gas upon dry and moist seeds respectively has been investigated by Townsend (34 and 35); the necessity for the investigation arose from the rapidly increasing use of this gas in the destruction of insect pests infesting stored grains and other seeds and also for the fumigation of greenhouses. Townsend found that *dry* seeds of corn, wheat, beans and clover might remain without injury to their germinating power for several weeks in an atmosphere containing a greater concentration of hydrocyanic acid gas than is required to kill quickly insect life. The germination of these seeds was found to be accelerated after this treatment and the rate of growth of the seedlings to be above the normal.

The action of boric acid upon the germination and subsequent development of seeds has been investigated by Morel (24). This author's results showed that brief treatment with boric acid at the time of germination produced a persistent deleterious effect upon haricot beans. The results noted about 7 weeks after sowing in ordinary soil are given in the following table:

TABLE X.

Treatment of the seeds previous to sowing in soil: Strength of acid used	Treatment of the seeds previous to sowing in soil: Period of soaking	Results noted in the plants after 7–8 weeks' growth
1 % boric acid	15 mins.	Showed no appreciable difference from the controls
1 % ,,	30 ,,	Differed from the controls only in the colour of their leaves which were of a less clear green
1 % ,,	60 ,,	The leaves of these plants were small and yellowish

It is seen that treatment of the *dry* seed with a 1 per cent. solution of boric acid even for ½ hour has a clearly visible effect upon the resulting plant during its whole course of growth.

In a similar series of experiments with seeds which had been previously immersed for 6 hours in water in order to render them more permeable to the acid solution, the following results were obtained:

TABLE XI.

Treatment of the seeds subsequent to the 6 hours soaking in water, but previous to sowing*		Results noted in the plants after about	
Strength of acid used	Period of soaking	5 weeks' growth	7 weeks' growth
1 % solution of boric acid	1 hour	Plants poor, with small yellowish leaves	Growth of plants moderately vigorous, but their leaves remained yellowish and were much smaller than those of the controls
1 % ,,	2 hours	Plants poor, with small yellowish leaves	
1 % ,,	3 ,,	A very few plants came up and these were very feeble	
1 % ,,	6 ,,	Few very feeble etiolated plants produced, leaves very small and few in number	Growth very poor although showing a decided improvement

* The seeds were placed on damp sand for a few days at a favourable temperature and were then sown in garden soil. Only those treated for the shortest period (*i.e.* 1 hour) had germinated after an interval of 3 days.

Similar experiments carried out with wheat gave results analogous to those obtained with haricot beans.

The present authors, working with *Brassica alba*, have shown that concentrations of carbonic acid (19) and boric acid (41) which rapidly kill the growing root, have no injurious effect upon any part of the embryo in the ungerminated but fully swollen seed. We may distinguish three ranges of increasing concentration of these two acids, as follows. (1) A range of low concentration in which the seeds germinate, but suffer injury after germination. (2) A range of intermediate concentration in which the germination of the seeds is inhibited. No injury to any part of the embryo or seedling can be observed even after prolonged treatment with this concentration of acid when the seeds are finally brought to germination in an acid-free medium. In this region marked injury is shown, however, by seeds which are allowed to germinate before being placed in the acid medium. (3) A range of higher concentration in which the seeds suffer injury whether ungerminated or germinated. The concentration of acid used by Morel lies in this third region which causes injury to the ungerminated seed.

This difference between the effect of chemical solutions upon the growing plant and their effect upon the seed before germination is not confined to acids but appears to be general. For instance, Hicks (13 and 14), who tested the effect upon seeds of a number of chemical fertilisers,

reached the conclusion that the effect of treating seeds with various chemicals before sowing is no index of the action of these chemicals when applied to the soil. Injury to the young plant may result when the chemicals are applied to the soil although no injurious effect is shown when the seeds are treated before germination with the same solution. However, little can be said on this subject with certainty until it has been determined whether the solute under consideration penetrates the testa or not.

(*b*) CHEMICAL AGENTS OTHER THAN ACIDS.

(I) *Copper Sulphate* (= *Bluestone*).

We have previously dealt with the effect of soaking seeds in water (18), and have drawn attention to the distinction which must be made between seeds sown wet (*i.e.* immediately after the soaking treatment) and seeds sown after re-drying, with or without storage. It is necessary to bear these points in mind when dealing with the effect of treating seeds with chemical solutions in which they are immersed for various periods.

In 1904 Bréal and Giustiniani (6) published the results of an investigation on the effect of treating the seeds of various cereals with a solution of copper sulphate. Unfortunately, although they appear to have been aware of the distinction which must be made between the effect of soaking and the effect of the solute alone, their results, whilst demonstrating a considerable increase in yield as a result of the treatment they employed, do not allow us to draw any critical conclusion as to how far they are due to the soaking and how far they are due to any specific action of the copper.

Their line of thought appears to have been as follows: Seeds sown after being allowed to take up moisture are found to give a greater yield, but damp seeds are very susceptible to fungal attack. Copper sulphate, however, has frequently been employed as a fungicide in seed-treatments. Hence, can seeds be soaked in a solution of copper sulphate, not only without injury, but with a result beneficial to yield? Further, might it not be advantageous to make up the copper sulphate solution together with 2–3 per cent. of starch, on the grounds that by so doing the escape of soluble food-materials from the seed into the surrounding medium during the period of soaking would probably be decreased[1]? They

[1] We have not found any published evidence to the effect that starch does decrease the exosmosis of soluble food-materials from the seed.

therefore adopted a Copper-Starch soaking treatment followed by liming and re-drying, and record results from which it appears that this treatment of the seeds renders them more resistant to fungal attack without affecting their germinating capacity. Moreover, seeds thus treated give rise to plants which are better developed and produce a larger yield than plants from untreated seeds. Bréal and Giustiniani drew attention to the fact that from the very beginning of their development the plants from the treated seeds gained on the controls, and that the superiority of the yield from the experimental plants over that from the controls was most marked in the case of the "heads." In the two following tables (Tables XII and XIII) the results of the authors' experiments are summarised, but these results, although of general economic interest, are unsatisfactory from our point of view because they do not show conclusively that the copper sulphate has any specific action on the seeds. Taken in conjunction with the beneficial results obtained by simply soaking cereal seeds in water, and in conjunction with the harmful effects of copper-treatments upon seeds recorded by other authors, it seems probable that the improvement of the plants following the copper-starch treatment of the seeds is to be attributed to the soaking alone.

TABLE XII.

Pot-culture experiment with equal weights of treated and untreated seed.

For details of the seed-treatments the original paper should be consulted.

Kind of seed	Duration of culture in days	Weight of the aerial parts of plants from treated seed, that of the control plants being taken as 100
Maize "Quarantain"	65	160
Wheat "Chiddam"	38	122
Barley "Chevalier"	36	120
Oats	35	110
White Lupin	33	119
Buckwheat	30	116

In a later investigation Bréal (5) confirmed his previous results and also conducted a number of water-culture experiments with treated and untreated seeds respectively of wheat, oats, barley and maize. These seeds were sown in vessels containing pure water, and the dry weight of the seedlings was taken after various short periods (*i.e.* 25–52 days). It was found (Table XIV) that in each case the total dry weight of the seedlings at the end of the experiment was less than that of the seeds originally sown, but that the plants from the treated seeds weighed considerably more than those from the untreated, this result

being due to the more vigorous stem and leaf development of the experimental plants. The results also show that the superiority of the plants from the treated seeds is evident from the beginning of their development.

TABLE XIII.

Experiments with equal weights of treated and untreated seeds sown "en pleine terre."

Maize seed used in each experiment.

No. of expt	Seeds	Fresh weight in kilograms after 100 days' growth		Weight of the aerial parts of the plants from treated seeds, that of the control plants being taken as 100	
		Entire yield	Heads	Entire yield	Heads
I	Treated	0·765	0·270	137	146
	Control	0·565	0·185		
II	Treated	3·100	0·920	120	129
	Control	2·500	0·710		
III	Treated	4·200	1·295	107	148
	Control	3·900	0·870		
IV	Treated	2·000	0·615	74	112
	Control	2·700	0·545		
V	Treated	4·000	0·605	114	121
	Control	3·500	0·500		

TABLE XIV.

Kind of seed	Duration of experiment in days	Dry weight in grams of the seeds sown	Dry weight in grams of yield from		% difference in yield in favour of the plants from treated seeds
			Untreated seeds	Treated seeds	
Wheat "Bordeaux"	25	88	60	72	20
,, ,,	52	88	45	66	46
Wheat "Dattel"	20	87	77	81	5
,, ,,	40	87	65	75	15
Wheat "Japhet"	20	89	77	80	4
,, ,,	40	89	60	67	11
Wheat "Bordier"	23	87	74	76	2
,, ,,	37	87	60	69	15
Wheat "Saumur"	20	88	75	83	10
Oats "Houdan"	27	87	70	78	11
Oats "Brie"	28	89	67	82	7
Barley	23	90	62	70	14
Maize "Gros jaune"	50	89	72	80	11
Maize "Dent de cheval"	32	89	73	81	10

Beyond the really important fact thus brought out that the final result can be forecasted at a very early stage in the development of the

seedling, it is impossible on the basis of the data given to determine the factors responsible for the results obtained. Bréal, however, is satisfied to conclude that the treatment ensures a better utilisation of the seed-reserves.

In view of the results obtained by Bréal and Giustiniani, which, as we have pointed out, cannot be attributed in the absence of further evidence to the specific action of the copper, the recent critical work of Jungelson (17), who worked with maize, is of interest. Jungelson used simple solutions of various copper salts and obtained similar results whatever the nature of the anion, thus definitely indicating that copper is the main factor in producing the results. He also showed that the results varied with the concentration of the salt.

The effects of the copper treatment on the seeds showed themselves throughout the whole course of development and were even carried over into succeeding generations. These effects, however, although very distinct and characteristic, were altogether harmful from the point of view of vigour and yield. Firstly, the copper treatment of the seeds decreases their power of germination, and secondly, there is both a retardation of the vegetative development and a delay in the flowering of the plants produced. Thirdly, a tendency towards variation, which manifests itself in the appearance of abnormal "heads" and seeds, is shown by the plants from the treated seeds; but these new characters do not appear to be handed on to the next generation. Lastly, plants from seeds treated in identically the same way exhibit totally different abnormalities.

Jungelson's results are especially important from two points of view; firstly, they demonstrate the persistence of the effect of the copper treatment of the seeds, and secondly they prove that the results of treating seeds with copper are distinctly unsatisfactory. Jungelson himself suggests that the degeneration of local races of cereals, which has become so marked of late years, may in a certain measure be due to the general use of seed-treatments in which copper is employed for the purpose of preventing the development of smut.

(II) *Other salts.*

We have already indicated the possibility that such results as those obtained by Bréal and Giustiniani (6) may be due to the soaking involved in their treatment of the seed rather than to the action of the copper. This possibility must be borne in mind when the question of other seed-treatments which involve soaking for certain periods in solutions of various salts is under consideration.

Accounts of the treatment of seeds with solutions of various salts with the object of testing the effect upon final yield are to be found scattered through the literature, but although sweeping conclusions are occasionally drawn, the experimental data upon which these conclusions are based are in the majority of cases completely inadequate. Two or three examples may be given.

In discussing the results of a long series of pot-culture experiments extending over a number of years, carried out at the Woburn Experiment Station with the primary object of testing the manurial value of small quantities of the so-called rarer constituents of ash, Voelcker (40) observes (p. 325) that "certain metallic salts have either a toxic or a stimulating effect upon vegetation, the particular effect depending upon the quantity of the metal present. At what stage of the plant's life are these influences exerted? The evidence so far adduced, leads strongly to the belief that it is during the germination of the seed, rather than at the later stages of the plant's growth, that these influences are exercised."

The substances tested by Voelcker were in most cases applied to the soil, but in a few experiments the only application of the substances was to the seeds, which were soaked in solutions for a short time before sowing. It was found that in some cases this treatment gave very favourable results, and in other cases it produced a moderately good result, whereas when the same substances were applied to the soil in various amounts, the results obtained were always negative. Some of the results obtained by Voelcker (37, 38, 39, and 40) are collected together in the following table (Table XV).

TABLE XV.

Kind of seed used	Treatment* Solution used	Period of soaking	Yield result expressed as % increase or decrease as compared with the control Grain	Straw
Winter Wheat (White Chaff Browick)	1 % NaI	10 mins.	+16·5	+30·1
	1 % NaBr	10 ,,	+ 8·7	+10·0
Wheat	10 % MnI	15 ,,	−30·9	− 4·5
	5 % MnI	15 ,,	− 0·4	+ 3·4
	1 % MnI	15 ,,	+ 3·1	+13·3
Barley	10 % NaBr	10 ,,	+13·8	+19·6
	10 % MnI	15 ,,	+12·7	+14·2
	5 % MnI	15 ,,	+ 5·6	+ 8·5
	1 % MnI	15 ,,	+14·2	+13·0

N.B. In a later series of experiments it was found that soaking the seed for 20 mins. in NaI or NaBr (1, 10, and 20 % solutions) did not give beneficial results.

* All the seeds were steeped in hot water for 10–15 minutes before treatment in order to kill smut.

These results should be accepted with considerable reserve since the pot-culture experiments were conducted with relatively few plants and the probable errors of the experiments were not determined.

A regular increase in yield with increased strength of the solution employed was reported by J. Craig (7) as the result of soaking pea seeds for one hour in solutions of $NaNO_3$ of the following concentrations, viz. 1 oz., 2 oz. and 3 oz. of the salt to one gallon of water.

One more instance may be noted. An increase in the tillering of several varieties of wheat to the extent of 12·8 per cent. and 20·6 per cent. respectively as the result of treating the seed with $(NH_4)_2SO_4$ (3 per cent. solution) and NH_4NO_3 (3 per cent. solution) was recorded by Wild (42) in the *Agricultural Journal of New Zealand*, 1914. In order to estimate the tillering 20 plants were taken at random from the various experimental plots, and in the case of each variety tested it was found that the effect of the treatment upon the tillering of the plants was a positive one. But here again the experimental data upon which the conclusions were based appear to be totally inadequate.

(III) *Hydrogen peroxide.*

Several workers have recorded observations on the effect of hydrogen peroxide treatments of seeds upon germination and upon the seedlings produced from the treated seeds. It would appear that in some cases hydrogen peroxide in certain concentrations may stimulate germination (cf. Pinoy and Magrou (28)), but the usual effect recorded is a retardation of the germination of the seeds.

Massee (22) when testing the effect of hydrogen peroxide upon seeds with a view to its possible utilisation in seed-sterilisation found that although germination was retarded, the growth of the seedlings eventually produced was rapid so that in many cases at the end of three weeks the plants from the treated seeds were distinctly larger than those from untreated seeds used as controls. But unfortunately the records of investigations with hydrogen peroxide are usually completely unsatisfactory for the reason that the strength of the solution employed is not determined.

CONCLUSIONS.

In concluding this review of the literature bearing upon what we have termed physiological pre-determination we may briefly summarise the available evidence. The evidence, as a whole, seems to show that

the factors which influence the plant during its earliest stages of development, have a more or less pronounced effect upon the whole of its subsequent life-history.

In the first chapter of this review we dealt with the factors which acted upon the plant whilst still a seed upon the parent. The most useful criterion here was found to be the size of the seed. A large amount of work bearing upon the effect of the size of the seed upon the growth and yield of the plant produced was reviewed, and an endeavour was made to distinguish critically between genetic and physiological factors. A certain amount of evidence was found indicating that the effects of the parental environment of a seed were sometimes only visible in the resulting plant.

In chapter II we dealt with the influence of the degree of maturity of the seed at the time of harvesting upon its "potentiality," and came to the conclusion that all comparisons made between mature and immature seeds were vitiated by the fact that immature seeds deteriorate more rapidly under storage conditions than mature seeds. In many cases it was found that the yield per plant from immature seed was at least equal to that from mature seed.

In the third and present chapters we have dealt with the aspect of the subject which is probably of most interest to the practical man, namely, the effect of the conditions during germination and in the early seedling stage upon subsequent growth and final yield. The work dealing with the soaking of the seed in water or salt solutions was critically reviewed in chapter III. In the present chapter the most interesting group of facts which have come under review appear to be those brought out by the work of Gassner and others on the effect of low temperatures in pre-determining the time of flowering of spring-sown winter cereals. This effect of exposure to low temperatures is a very clear and distinct phenomenon. While it still remains unexplained, it nevertheless bears out the general thesis based on the evidence which we have collected from various sources, namely, that the external conditions which obtain during the early stages of the development of the plant have a very pronounced effect upon its subsequent development. The results of Gassner's investigations show clearly that it is during the first few hours of germination that the "pre-determination phenomenon" can be most easily and quickly brought about by exposure to cold. As the seedling develops, the duration of the exposure to cold and the degree of cold necessary to ensure flowering in the same year rapidly increase.

While much is known with regard to the effect upon germination of

various chemical treatments of seeds, very little experimental work is on record with regard to the subsequent growth and yield of the plants produced from the treated seeds. All the available evidence, however, supports the conclusion that where germination and early seedling growth are stimulated by chemical treatments of the seed the subsequent growth and final yield are favourably influenced in proportion.

One of the most interesting outcomes of the present review of literature has been to emphasize the fact that normal plant-growth falls into line with a "compound interest" law of development. The data obtained by various workers from growth experiments with plants from seeds which had been deprived of part of their original food-reserves show that, broadly speaking, the growth and yield of the resulting plants are proportional to their initial "food-capital," and thus provide an illuminating demonstration of this "compound interest" law.

BIBLIOGRAPHY.

(1) Appel, O. und Gassner, G. Der schädliche Einfluss zu hoher Keimungstemperaturen auf die spätere Entwicklung von Getreidepflanzen. *Mitteil. aus d. Kais. Biolog. Anst.* and *Land- und Forstwirtschaft*, Heft 4, 1907, p. 5.

(2) Atterberg, A. Die Nachreife des Getreides. *Landw. Versuchs-St.* 67, 1907, p. 129.

(3) Babcock, S. M. Metabolic Water: Its Production and Rôle in Vital Phenomena. *Univ. of Wisconsin Agric. Expt. Sta., Research Bull.* No. 22, March, 1912.

(4) Bokorny, T. Ueber den Einfluss verschiedener Substanzen auf die Keimung der Pflanzensamen: I, II and III. *Biochem. Zeitschr.* 50, 1913.

(5) Bréal, E. Traitement Cuivrique des Semences. *C. R. de l'Acad. des Sc.* Paris, 142, 1906, p. 904.

(6) —— et Giustiniani, E. Sur un Nouveau Traitement des Semences. *C.R. de l'Acad. des Sc.* Paris, 139, 1904, p. 554.

(7) Craig, J. Rpt. of the Horticulturist. *Canada Expt Farms Rpt.*, 1897.

(8) Fischer, A. Wasserstoff- und Hydroxylionen als Keimungsreize. *Ber. d. Deutsch. Bot. Ges.* 25, 1907, p. 108.

(9) Gassner, G. Beobachtungen und Versuche über den Anbau und die Entwicklung von Getreidepflanzen im subtropischen Klima. *Jahresber. d. Vereinig. f. Angew. Bot.* 8, 1910, p. 95.

(10) —— Beiträge zur physiologischen Charakteristik sommer- und winterannueller Gewächse, inbesondere der Getreidepflanzen. *Zeitschr. für Bot.* 10, 1918, p. 417.

(11) —— und Grimme, C. Beiträge zur Frage der Frosthärte der Getreidepflanzen. *Ber. d. Deutsch. Bot. Ges.* 31, 1913, p. 507.

(12) Goodspeed, T. H. Notes on the Germination of Tobacco Seed. *Univ. of California Publications in Botany*, v, No. 5, 1913, pp. 199–222.

(13) HICKS, G. H. The Effect of Fertilizers on the Germination of Seeds. *Proc. Amer. Assoc. Advanc. Sc.* 47, 1898, p. 428.

(14) —— The Germination of Seeds as Affected by Certain Chemical Fertilizers. *U. S. Dept. Agric. Bot. Bull.* 24, 1900.

(15) HÖSTERMANN. Versuche über die Beeinflussung des Erntenutzungswertes durch die "Elektrokultur." *Landw. Jahrb.* 45, I, 1913, p. 77.

(16) HOTTER, E. Ueber die Vorgänge bei der Nachreife von Weizen. *Landw. Versuchs-St.* 40, 1892, p. 356.

(17) JUNGELSON, A. Sur des Épis anormaux de Maïs obtenus à la suite du Traitement Cuivrique de la Semence. *Rev. gén. de Bot.* 29, Nos. 344 and 345, 1917.

(18) KIDD, F. and WEST, C. Physiological Pre-Determination: The Influence of the Physiological Condition of the Seed upon the Course of Subsequent Growth and upon the Yield. IV. Review of Literature. Chapter III. *Ann. Appl. Biol.* 5, 1919, p. 220.

(19) —— The Controlling Influence of Carbon Dioxide. Part IV. On the Production of Secondary Dormancy in Seeds of *Brassica alba* following Treatment with Carbon Dioxide, and the Relation of this Phenomenon to the Question of Stimuli in Growth Processes. *Ann. of Bot.* 31, 1917, p. 457.

(20) KINZEL, W. Frost und Licht als beeinflussende Kräfte bei der Samenkeimung, etc. Stuttgart, 1913.

(21) KRAŠAN, F. Beiträge zur Physiologie der Pflanzen. *Sitzungsber. d. Kais. Akad. d. Wiss. math.-naturwiss. Cl.* Wien, 68, I Abt. 1874, p. 195.

(22) MASSEE, I. The Sterilization of Seed. *Journ. Board of Agriculture* (Lond.), 20, 1913–1914, p. 796. Also in *Kew Bull.* 5, 1913, p. 183.

(23) MICHEELS, H. et DE HEEN, P. Action des Courants Alternatifs de Haute Fréquence sur la Germination. *Bull. de l'Acad. Roy. de Belgique* (*Cl. des Sc.*), 1908, p. 82.

(24) MOREL, J. Action de l'Acide Borique sur la Germination. *C. R. de l'Acad. des Sc.* (Paris), 114, 1892, p. 131.

(25) OCKEL, E. *Bericht über das Versuchsfeld Frankenfelde*, Berlin, 1854, p. 147.

(26) ONODERA, I. Untersuchungen über die Beschädigung der Pflanzen durch Säuren und über die Reizwirkungen der Säuren auf Pflanzen. *Ber. d. Ōhara Inst. f. landw. Forsch.* 1, I, 1916, p. 53.

(27) PIETRUSKY. Versuch mit gedörrtem Leinsamen. *Landw. Jahrb.* 2, 1873, p. 138.

(28) PINOY et MAGROU. Sur la Stérilisation des Graines. *Bull. de la Soc. Bot. de France*, 59, 1912, p. 609.

(29) PLATE, F. Ricerche sui fenomeni d' imbibizione dei semi di *Avena sativa. Atti della Reale Acad. dei Lincei Rendiconti*, 22, 1913, p. 133.

(30) PROMSY, G. *Du Rôle des Acides dans la Germination.* Marseille, 1912.

(31) —— et DREVON, P. Influence des Rayons X sur la Germination. *Rev. gén. de Bot.* 24, 1912, p. 177.

(32) SIGMUND, W. Ueber die Einwirkung chemischer Agentzien auf die Keimung. *Landw. Versuchs-St.* 47, 1896, p. 1.

(33) SPRENGEL, C. *Meine Erfahrungen im Gebiete der allgemeinen und speziellen Pflanzenkultur*, Bd. 1, Leipzig, 1847, p. 86.

(34) TOWNSEND, C. O. The Effect of Hydrocyanic Acid Gas upon the Germination of Seeds. *Proc. Amer. Assoc. Advanc. Sc.* Columbus, 1899, p. 297.

(35) Townsend, C. O. The Effect of Hydrocyanic Acid Gas upon Grains and Other Seeds. *Bot. Gaz.* 31, 1901, p. 241.

(36) Velten, W. Ueber die Folgen der Einwirkung der Temperatur auf die Keimfähigkeit und Keimkraft des Samen von *Pinus Picea* Du Roi. *Sitzungsber. d. Kais. Akad. d. Wiss. math.-naturwiss. Cl.* Wien, 74, I Abt. 1876, p. 359.

(37) Voelcker, J. A. Woburn Pot-Culture Experiments; The Hills Experiments. *Journ. Roy. Agric. Soc.* England, 61, 1900, p. 553.

(38) —— Woburn Pot-Culture Experiments; The Hills Experiments. *Journ. Roy. Agric. Soc.* England, 62, 1901, p. 317.

(39) —— Woburn Pot-Culture Experiments; The Hills Experiments. *Journ. Roy. Agric. Soc.* England, 64, 1903, p. 348.

(40) —— Woburn Pot-Culture Experiments; The Hills Experiments. *Journ. Roy. Agric. Soc.* England, 73, 1912, p. 314.

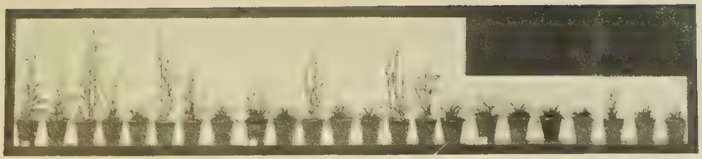
(41) West, C. and Kidd, F. The Controlling Influence of Carbon Dioxide. Part V. On the Production of Primary and Secondary Dormancy in Seeds of *Brassica alba* by means of Boric Acid, and the Relation of Hydrogen-ion Concentration to the Production of Secondary Dormancy. (In the Press.)

(42) Wild, L. J. The Tillering of Wheat. *Journ. of Agric.* (New Zealand), 9, 1914, p. 31.

(43) Wollny, E. Das Dörren der Samen. *Oesterr. landw. Wochenblatt*, 5, No. 48, 1879.

(44) —— *Saat und Pflege der landwirtschaftlichen Kulturpflanzen.* Berlin, 1885.

I.



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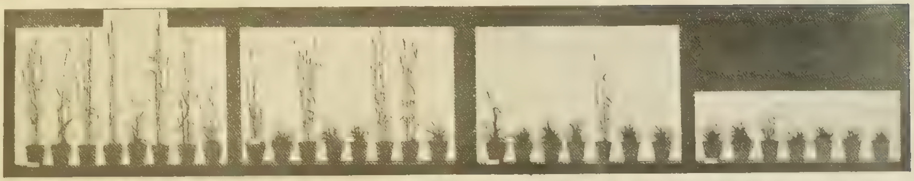

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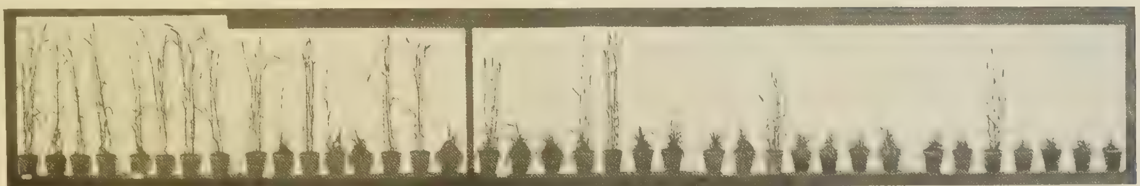

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March April May June

The development of "Petkuser" Winter Rye in relation to the germination temperature and the time of germination (after Gassner).

Fig. I was photographed on June 8th. Fig. II was photographed on June 27th. Fig. III was photographed on July 26th.

STUDIES IN BACTERIOSIS. III.

A BACTERIAL LEAF-SPOT DISEASE OF *PROTEA CYNAROIDES*, EXHIBITING A HOST REACTION OF POSSIBLY BACTERIOLYTIC NATURE.

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(With Plate II.)

THE disease was observed on plants of *Protea cynaroides* in the houses at Kew Gardens where, for a number of years—practically since the introduction of this species to the Gardens—it has caused considerable disfigurement to the plants. It occurs on the leaves of all the older plants and shows itself on the leaves of seedlings when these have reached a height of some 10 or 12 inches.

SYMPTOMS OF THE DISEASE.

The disease is characterised by numerous dome-shaped blisters of a reddish-brown colour scattered promiscuously over the lamina of the leaf, mainly, though not entirely, upon the upper surface. They vary in diameter from one to three millimetres and the surface of the blisters is raised half to one millimetre above the general level of the leaf. On the younger leaves in place of the brown blisters there occur rather wider areas whose surface is frequently depressed by shrinkage of the underlying cells. These areas have a diameter up to five or six millimetres, and still larger patches arise through the coalescence of several such spots. The colour of these depressed areas is much brighter than that of the blister-like spots and is either a uniform red or a reddish-brown surrounded by a zone of bright vermilion. The vermilion colour is very conspicuous when the leaf is held up to the light. On examination in this way by transmitted light every spot exhibits a clear translucent halo in a zone of one or two millimetres round the spot. The appearance of the diseased

tissue and the nature of the cell contents are identical in the raised and depressed areas; and, as will be described later, an organism has been isolated from the dead tissue of one type of spot which on inoculation into a healthy leaf has given rise to a spot of the other type; there is therefore no doubt that both types of spot have a common origin. It is not known whether the one changes into the other, or whether they are the result of difference in age of the leaf at the time infection took place. Spots of both types occur on one and the same leaf and may be recognised in the photograph (Fig. 1 *A*); the sunken type with bright red colour (Fig. 1 *B*) is, however, the only one which has resulted from artificial infection of young seedling plants (Fig. 2). For these figures see Plate II.

Pathological Anatomy.

Microscopical examination of a section through one of the spots shows the cells completely disorganised, the normal contents having given place to a yellow or brown gum-like mass. In fresh material the mass completely fills the cavity of the cell, but in fixed material it is found to have contracted slightly from the walls of the cells (Figs. 4 and 5). The group of affected cells is frequently cut off from the surrounding tissue by a zone of three or four layers of cork cells. No fungal mycelium is present in the diseased tissue, and no bacteria can be found in the intercellular spaces. In many spots clear evidence of the parasitic origin of the disease is lacking, but in others one or two cells, usually epidermal cells, are found filled with granules which have all the appearance of micrococci and which take the bacterial stains strongly (Fig. 3). In these cells the gum-like substance is either absent altogether or is present in such small quantity that only a very faint yellow colour is observable. In the majority of the diseased cells, however, this substance is present, and exhibits a variety of differences in its minute structure and staining capacity which are thought to have considerable significance. An attempt to reproduce these differences by shading has been made in Figs. 4 and 5; the drawings are from a hand section through a "blister-spot," and the section was stained with carbol fuchsin and partially decolourised with 50 per cent. alcohol[1].

The substance in some cells retains the fuchsin strongly and includes a mass of closely compacted granules which stain still more strongly with fuchsin and other bacterial stains. Fig. 5 shows several such masses.

[1] The similarity in actinic value of the red and orange rays makes it impossible to demonstrate these differences of colour and structure by a photographic process.

In the cell marked *A* the gummy mass was densely stained and appeared very definitely granular, the substance surrounding the granular mass possessed a fine foam-structure. In cell *B* granules of about the size of small bacteria could be distinctly recognised; here also the matrix showed a foam-structure, but of a coarser character than in cell *A*. In cell *C* masses of densely staining material were surrounded by a mass

Fig. 3. Drawn with the aid of the camera lucida from a microtome section 4μ in thickness through a leaf spot of *Protea cynaroides*; note the very frangible nature of the gum-like substance filling the majority of the cells. *A*, cell filled with granules believed to be bacteria, the cause of the disease; *B*, cell filled with gum in which are embedded similar granules which appear to be in process of dissolution.

of gum which had become very vacuolate and which took up the fuchsin stain only weakly. Cell *D* was almost completely filled with similar vacuolate material. The cells which are shaded uniformly in Figs. 4 and 5 were filled entirely with structureless transparent gum-like material which showed different degrees of staining capacity in the different cells, as is shown, for instance, in the cells marked *A* and *B*, Fig. 4. The substance in cell *A* was entirely unstained by fuchsin and possessed a

clear bright amber colour, while in cell *B* it retained a fairly strong fuchsin stain. We have, then, what appears to be a series of stages in the disappearance of such granules as are represented in Fig. 3. The nature of these granules is not definitely established, but a micro-organism has been isolated from diseased tissue with which these bodies compare closely in size, and since bacteria in the diseased tissue are indicated in no other way and, moreover, the granules stain deeply

Fig. 4. Drawn with the aid of the camera lucida from a hand section through a "blister-spot" on the leaf of *Protea cynaroides*. The cells shaded darkly contain a more or less granular matter embedded in a gum-like matrix, and they retain a fuchsin stain in proportion to the degree of granulation. The cells shaded uniformly contain structureless transparent masses of gum which reacted variously with fuchsin, some, as cell *A*, lost all the stain and possessed a clear amber colour after treatment of the section with 50 per cent. alcohol, while neighbouring cells, as cell *B*, retained a fairly strong fuchsin stain.

with carbol fuchsin and Victoria blue, the assumption of their bacterial nature seems amply justified. It is not always possible to demonstrate in sections of diseased tissue such bacteria-like granules, but in all cases masses of the granular gummy substance occur in various degrees of granulation and of staining capacity, and the degree of granulation always runs *pari passu* with the power of retention of a bacterial stain. One is therefore led to assume that bacteria entering the cell are early

killed by some poisonous substance and eventually disappear by a process of solution, the vacuolation of the gum being interpreted as marking

Fig. 5. Portion of the section shown in Fig. 4, more highly magnified. *A*, cell containing a gum-like matrix with foamy structure and including a densely granular and deeply stained mass believed to represent bacteria in process of solution, the foamy nature of the matrix may possibly mark the presence of degradation products from similar granules. *B*, cell containing similar contents, the more open nature of the foam indicates perhaps a later stage in the process of degradation. *C*, cell containing what appear to be oily drops in the gum-like matrix. *D*, cell containing vacuolate gum in which bacteria-like granules can no longer be recognised. *E*, a cell similar to that marked *A*, but out of focus. *F*, cell *A* of Fig. 4, containing perfectly structureless amber coloured gum. *G*, cell *B* of Fig. 4, containing similar transparent gum but stained fairly strongly with fuchsin, a feature which is taken to indicate that some bacterial substance is still present in the gum.

the presence of certain degradation products of the bacterial cell. (There may possibly be an analogy here with the solution of a bacillus, as for

example of the cholera bacillus, in the bacteriolytic serum of an immunised animal.) This view is supported by the fact that in sections of some of the spots bacteria cannot be definitely recognised, and further by the fact that when attempts are made to isolate the parasite many of the spots appear to be sterile. A somewhat similar pathological condition has been described by Potter[1] in a brief account of a raised leaf-spot disease of an orchid, *Odontoglossum Uro-Skinneri*; the swelling of the tissue is there attributed to the production of a mucilaginous substance in which bacteria, believed to be the cause of the disease, are found embedded. It is possible that the substance is of the same nature in both cases, but in *Odontoglossum* it is described and figured as occupying the intercellular spaces while in *Protea* it is found only within the cells.

Some attempt was made to determine the nature of this substance in *Protea*, but unfortunately the amount of material available was too limited to permit of any extensive investigation of the part it plays in the bacteriolytic process or of its chemical composition. First it should be noted that the presence of this substance is not confined solely to the diseased areas, but appears in its structureless non-staining variety wherever the tissues are wounded and also in certain cells in the neighbourhood of the vascular bundles. It may perhaps be an ordinary tannin product of the host cells and, if the assumption of bacteriolysis is correct, may serve in the diseased area simply as a vehicle in which the solution of bacteria is brought about by some other agency, possibly by autolysis.

The gummy substance becomes very brittle in fixed material, and in consequence it has been found very difficult to prepare good sections of the diseased tissue, and almost impossible to obtain microtome sections. The irregularities and the fissures in the substance depicted in Figs. 3 and 5 give some idea of its mechanical texture; the material from which the sections here shown were prepared had been preserved in weak formalin.

Fig. 3 was drawn from a very imperfect microtome section typical of several attempts to cut the material on the microtome; with the exception of one of the epidermal cells only fragments of the tissue were obtained in spite of the fact that the cuticle, itself a difficult subject for the knife, was cut perfectly. The epidermal cell referred to, presumably recently invaded by bacteria, possessed a matrix of a scarcely perceptible yellow tinge, while the contents of many of the neighbouring cells were deep yellow to dark amber in shade[2]. This is taken to indicate that the

[1] *Gardeners' Chronicle*, Ser. III, vol. 45, p. 145. 1909.

[2] The bacterial stain employed in this case was Victoria blue, not fuchsin.

formation of the gummy substance had only just begun, hence the contents were so little refractory that a tolerable section of this cell was obtained.

In spots on young leaves the yellow material is accompanied by a bright vermilion pigment. This is insoluble in water and in alcohol, even after 24 hours steeping of the tissue in boiling alcohol; it is slightly soluble in ether; it is insoluble in hydrochloric acid but dissolves slowly in cold concentrated sulphuric acid; it is readily soluble in ammonia, from which it is precipitated as a red powder by hydrochloric acid. These reactions, with the exception of sparing solubility in alcohol, are those of phlorotannin red, but solutions of the red precipitate in caustic soda do not show the fluorescence characteristic of this substance (Beilstein, 3rd ed., vol. 2 B, p. 1919). A substance of this character would probably be sufficiently toxic to account for the death of the bacteria; their subsequent disappearance is, however, a matter of pure conjecture.

After extraction of the red pigment by ammonia the yellow substance remains apparently unaltered. It stains black with ferrous sulphate and may perhaps be of the nature of a resino-tannin.

Isolation of the Parasite.

The surface of diseased leaves was sterilised by immersion in hydrogen peroxide for one hour, after which the leaves were allowed to dry and the epidermis was removed from a young blister-spot by means of a flamed scalpel. A little of the exposed soft brown tissue was crushed in sterile water and dilution plates in bouillon gelatine and bouillon agar were poured Several attempts at isolation in this way failed to give any result; this may have been the effect of the treatment with hydrogen peroxide, but it is considered more probable that the organisms in the spots selected had been destroyed in the manner suggested above. Eventually, however, success was attained; numerous colonies appeared on the plates after three days incubation at 20° C. and were apparently all of one type. Repeated platings yielded pure cultures without much difficulty.

Infection Experiments.

Through the courtesy of the Director of Kew Gardens two small plants of *Protea cynaroides* were available for inoculation. These were sturdy young seedlings six and eight inches in height, and had several young leaves which were quite free from the disease. In all, fourteen experiments were made upon seven separate occasions, using seven

different cultures of the organism. The inoculations were made in various ways, and of the fourteen attempts only one failed to produce infection.

Experiment 1. May 29th, 1918. The surface of a leaf of a potted plant was sterilised by washing with alcohol and two inoculations were made by placing a spot of slime from an agar culture (the third transfer from the original isolation) upon the upper surface of the leaf and pricking through this into the leaf with a flamed needle; two controls were made by similarly pricking through slime which had been heated. May 31st: a translucent margin one millimetre wide had appeared round the punctures, and a mucilaginous drop was present above the point of infection; control spots had dried up. June 2nd: tissue was brown in a zone of 2 mm. radius round the prick; control pricks were dry and browned only at the edge of the needle puncture. June 8th: typically diseased spots; controls dried out. Plate cultures from a suspension of the diseased tissue showed on June 10th development of many colonies of two distinct types with a strong preponderance of that characteristic of the disease organism. A second plating from these gave pure cultures which were used in Experiment 3.

Experiment 3. June 12th, 1918. The organism re-isolated from the leaf of Experiment 1 was pricked into a cut leaf placed on moist blotting-paper in a Petri dish and kept at room temperature. June 17th: tissue was brown and typically diseased two millimetres round both infection spots and mucilaginous drops were standing above as in Experiment 1; control pricks had dried up. A suspension of the diseased tissue was employed in Experiment 7.

Experiment 7. June 18th, 1918. Diseased tissue from leaf of Experiment 3 was suspended in sterile water and pricked into a leaf of a growing plant. June 30th: all inoculated spots were typically diseased; all controls had dried up.

Experiment 4. June 14th, 1918. The organism from an agar slope (the fifth transfer from the original) was pricked into a cut leaf with its petiole immersed in sterile water and covered with a bell-jar. It was placed in strong light in the greenhouse laboratory. June 20th: around the two infection pricks the tissue was browned in a spot 4 mm. diameter and this was surrounded by a wider zone 6 or 7 mm. diameter in which the bright vermilion pigment typical of the disease in young leaves had developed abundantly. A photograph of this leaf appears in Plate II, Fig. 2.

Other infections were obtained by hypodermic injection of pure cultures, by placing bacterial slime upon the leaf surface without punc-

turing the leaf, and by gently rubbing with the finger a water suspension of the organism upon the upper surface of the leaf. Except in one case typical disease resulted from these experiments. Control experiments were made with heated bacterial slime and with a gum arabic solution brought to the opacity of the slime with potato starch. The object of these controls was to determine whether physiological influences, such as the blocking of the stomata or the local shading from light of the tissue, would produce the symptoms of disease. None of these controls showed any browning effect. The brown substance however is formed in response to wounding round the needle pricks in control experiments but, as seen in Plate II, Fig. 2, there is a marked difference in the extent of discolouration between the infection and the control spots.

The fact that infection could be produced by gently rubbing a suspension of the organism over the surface of the leaf would suggest that the mode of entry of the bacteria into the leaf under natural conditions is by way of the stomata. In accordance with the xerophytic habit of the plant the cuticle of the leaf, which is exceedingly thick, is raised above the guard cells of the stoma in the form of a spacious cup (Fig. 4) eminently suited to catch water and act as a port of entry for bacteria to the chamber below. The spots always appear to have developed in connection with the stomata, and in a large number of cases a single stoma has been found occupying the very centre of the surface of the diseased area, though no sections of diseased spots have revealed the presence of bacteria in either the stoma itself or in the sub-stomatal chamber.

DESCRIPTION OF THE CAUSAL ORGANISM.

(i) *Morphological Characters.*

Form and Size. The organism taken from an agar slope incubated for 24 hours at 22° C. is a small oval rod $0{\cdot}8$–$1{\cdot}6\mu \times 0{\cdot}6$–$0{\cdot}8\mu$; pairs up to $2{\cdot}5\mu \times 0{\cdot}8\mu$. In sections of diseased spots where organisms can be found they appear to be of about the same diameter but almost coccoid in form.

Motility. In young cultures the organism is actively motile with a free swimming motion. The flagella stain quite readily by the method of van Ermengen and a beautiful preparation was obtained by the method of Plimmer as yet unpublished[1]; they are from one to three in

[1] It is hoped that the publication of this method will not be long delayed.

number, 10–20μ in length and are unipolar (Fig. 6). The organism is therefore a *Pseudomonas.* Motility soon ceases in cultures on solid media, even after 24 hours at 22° C. only a few individuals are found in motion; in liquefied gelatine and in bouillon, however, motility was observed in cultures of three days incubation at the above temperature.

Staining. Positive results were obtained with the usual bacterial stains and with Gram's stain, negative results with spore stains and capsule stains.

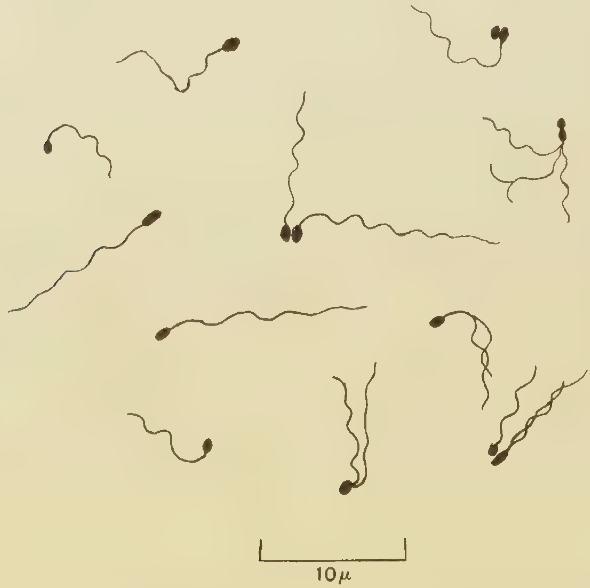


Fig. 6. Drawn with the aid of the camera lucida from a preparation of *Pseudomonas Proteamaculans* stained by the method of Plimmer (unpublished).

(ii) *Cultural Characters.*

The organism grows luxuriantly on solid media of various composition and of varying H ion concentration up to about + 30 of Fuller's Scale.

Gelatine streak + 10. Incubated at 22° C. Liquefaction 4 mm. wide and 2 mm. deep after 24 hours; complete liquefaction after 72 hours.

Gelatine stab + 10. Incubated at 22° C. Liquefaction at first crateriform, the crater being 5 mm. wide and 2 mm. deep after 24 hours; later, *i.e.* after 3 days, infundibuliform with a layer of liquid gelatine 6 mm. deep above, the funnel being 7 mm. across at the top and 3 mm. at the bottom.

Agar streak + 10. Incubated at 22° C. Streak from straight wire inoculation 2 to 1 mm. wide after 24 hours, margin entire, surface raised 0·5 mm., "wet shining," dirty white with faint yellow tinge, distinctly yellow when collected on a wire.

Agar stab + 10. Incubated at 22° C. Growth along the stab uniform to bottom with echinate margin, surface growth after 24 hours 3 mm. diameter and raised 0·5 mm.

Potato agar. Incubated at 22° C. Very strong growth after 24 hours. Characters as on bouillon agar but perhaps a trifle more strongly yellow tinted.

Bouillon + 10. Incubated at 22° C. Well clouded after 24 hours. Slight ring but no pellicle.

Thermal Death Point. Twelve tubes of bouillon were placed in a water-bath and raised to 47° C. registered on a thermometer with its bulb immersed in water in a similar tube. The tubes were inoculated in duplicate with a loopful of a bouillon culture of 24 hours growth. The temperature of the bath was raised 2° C. between the inoculation of each pair of tubes and the inoculated tubes were maintained at their appropriate temperatures for 10 minutes, then plunged into cold water. All were incubated at 25° C. for 7 days. The thermal death point was found to lie between 51° and 53° C.

(iii) *Physiological Characters.*

Bouillon ("*Jardox*") + 2 *per cent. sugar.* Acid and gas formed in presence of glucose, sucrose and mannite. The amount of gas was not large and occupied only one-tenth of the volume of a Durham's tube after 7 days incubation at 22° C., and was approximately the same in each case. A slight ring formed, but no pellicle. Bleaching of the litmus occurred at the bottom after 4 days. No acid nor gas formed in lactose bouillon though growth was indicated by slight turbidity and ring formation.

Bouillon ("*Jardox*") + 1 *per cent. nitrate.* Nitrite was present after 24 hours at 22° C. and was still present after 2 months, at which time, by transferring to other media, the organisms were shown to be still viable.

Potato plug. Growth well visible after 24 hours at 22° C., and strongly developed after 48 hours, faint yellow in colour; the surrounding medium was not discoloured. The plug ground in a mortar after 12 days and suspended in 300 c.c. of water gave, when tested with iodine, a more purple colour than a control plug, showing slight diastatic action had occurred.

Uschinsky's solution. No acid and no gas; strong ring and no pellicle.

Litmus milk. Acid after 24 hours at 22° C., loose curd after 2 days, and whey separated after 4 days, whey clear and colourless; curd settled to half the volume of the liquid after 7 days, and still occupied one-third of the volume after 2 months.

Nomenclature.

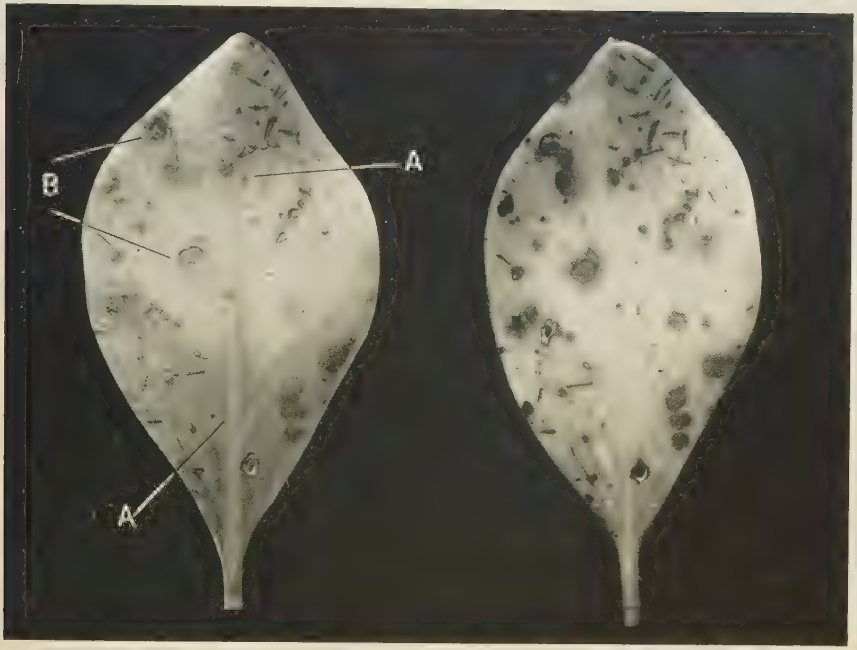
The organism is a pseudomonas and does not agree with any previously described organism. It is therefore believed to be a new species and the name *Pseudomonas Proteamaculans* is suggested for it. According to the system of the American Society of Bacteriologists it is represented by the number 221.1313023.

Control Measures.

In the present state of our knowledge the control of bacterial diseases is very difficult. In this instance the bacteria gain entrance to the leaf by way of the stomata and are undoubtedly introduced as a result of syringing the plants. The presence of some antiseptic in the water used for syringing which shall be toxic to the organisms and harmless to the plant therefore suggests itself as a possible means of preventing infection. With this end in view the following experiments were made. Test tubes containing 10 c.c. of the solutions given below were sterilised and inoculated with a loopful from an active culture of *Pseudomonas Proteamaculans*, and after the time stated in each case a loopful was transferred to bouillon agar and plated out. The number of colonies developed at 20° C. was determined after three days incubation.

No.	Solution	Time of exposure	No. of colonies
1	·001 % $HgCl_2$	1 min.	200–300
2	„	5 „	none
3	„	10 „	„
4	„	15 „	„
5	$\frac{1}{400}$ formalin	1 „	∞
6	„	5 „	100–200
7	„	10 „	none
8	„	15 „	„
9	·001 % $CuSO_4$	1 „	∞
10	„	5 „	∞
11	„	10 „	90
12	„	15 „	40

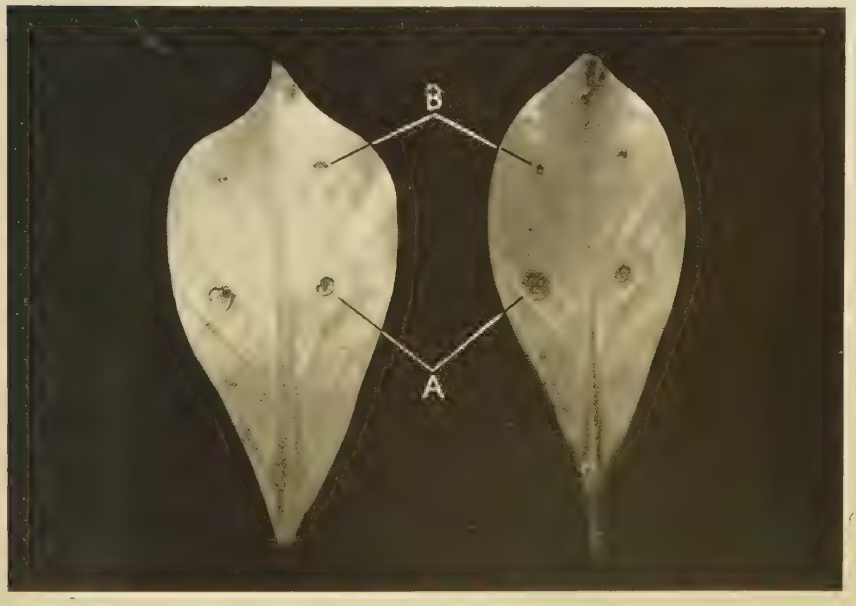
From this it appears that a solution of mercuric chloride (1 : 10,000) is most efficient, but, in view of the poisonous nature of this substance,



(a) Fig. 1. (b)

(a) Diseased leaf of *Protea cynaroides* showing two types of bacterial spots, raised blister-like spots at *A* and sunken spots at *B*.

(b) The same leaf photographed through an orthochromatic light filter.



(a) Fig. 2. (b)

(a) Leaf of *Protea cynaroides* artificially infected by means of "prick" inoculations with a pure culture of the causal organism. *A* infection spots; *B* controls.

(b) The same leaf photographed through an orthochromatic light filter.

the formalin solution (1 : 400) would be much safer and would probably have the desired effect.

SUMMARY

1. A description is given of a disease of *Protea cynaroides* which is characterised by the development on the leaves of blister-like spots of a brown colour, or of sunken spots of a brown colour with bright vermilion border.

2. The causal organism described is a bacterial parasite for which the name ***Pseudomonas Proteamaculans*** is suggested.

3. The attacked cells become filled with an amber coloured resin-like substance in which apparently the bacteria become embedded and suffer a process of solution. This solution is either one of autolysis, or there may possibly be an analogy with the bacteriolytic action of the serum of an immunised animal.

4. The vermilion pigment is shown to be closely allied to phlorotannin red and the resin-like substance is believed to be of the nature of a resino-tannin.

ON THE OCCURRENCE OF THE IMMATURE STAGES OF ANOPHELES IN LONDON.

By FLORENCE E. JARVIS.

The observations recorded below were made between September 1917 and September 1918. The area selected was approximately a circle with its centre at Charing Cross and a radius of about nine miles. The majority of the pieces of water examined were ornamental ponds in the various London parks, but in addition a number of natural ponds, swamps, and ditches were searched.

Positive results for *Anopheles maculipennis* were obtained from sixteen out of a total number of thirty-seven pieces of water investigated. *A. bifurcatus* occurred in one locality; *A. plumbeus* (= *nigripes*) was not observed.

During the spring and early summer of 1918 the presence of immature stages of *A. maculipennis* was noted in or near the outer limit of the selected area only. Later in the season, in August, considerable numbers of larvae were obtained from places much nearer the centre of the circle, *e.g.* Chelsea Physic Garden and Battersea Park. These facts seem to suggest the possible occurrence during the summer of an inward migration of adults from more outlying suburbs. The two places mentioned above lie opposite one another on the north and south sides of the Thames respectively and are enclosed by houses on three sides, the fourth side being open to the river. A migration of adults to Chelsea and Battersea could thus conceivably take place along the course of the river from the marshy districts lying near the mouth. The west and south-west winds however, which prevailed during the greater part of the summer of 1918, would appear to have been able to hinder effectively a migration in this direction. A visit made in September to an ornamental pond in Staple Inn yielded a negative result which could be attributed (1) to the densely-populated nature of the locality, and (2) to the presence of a number of gold-fish in the water.

In the accompanying table are given, in order of date, the pieces of water visited. Many of these proved unsuitable for breeding places, while in certain other cases conditions were apparently favourable but

negative results were obtained. Further investigation of these apparently favourable localities is desirable.

Since the presence of larvae of *A. maculipennis* has now been observed as far within the urban district of London as Chelsea, records are needed of the occurrence of adults in this area. Up to the present time adults have only been recorded from suburban localities (Acton, Mitcham, etc.) all lying at a considerable distance from Charing Cross.

In order to make this report as complete as possible, previous records have been incorporated into it. All such records have been drawn from W. D. Lang's *Map showing the known Distribution in England and Wales of the Anopheline Mosquitoes, with explanatory Text and Notes*, published by the British Museum (Natural History), 1918. The Local Government Board's *Reports and Papers on Malaria contracted in England in* 1917 (New Series, no. 119, 1918) has also been consulted, but contains no additional information on the London area.

The writer is extremely indebted to Mr W. D. Lang for his kindness in identifying numerous larvae and adults, to Mr A. J. Grove (Acting Entomological Investigator, Local Government Board), and to Mr Hugh Scott for his assistance throughout the course of the investigations.

TABLE SHOWING PLACES VISITED, IN ORDER OF DATE.

Date	Locality	*A. maculipennis*	*A. bifurcatus*	
28. ix. 1917	Richmond Park	*	—	
2. x. 1917	,, ,, ...	*	—	
19. x. 1917	Swamp near Southend Pond, Catford, S.E.	—	*	
26. x. 1917	,, ,, ,, ,,	—	*	
30. x. 1917	,, ,, ,, ,,	—	*	
2. xi. 1917	Regent's Park	—	—	
23. xi. 1917	Swamp near Southend Pond ...	—	*	
27. xi. 1917	Chelsea Physic Garden	—	—	†
28. ii. 1918	Horniman's Museum, Forest Hill, S.E.	—	—	
28. ii. 1918	Dulwich Park, S.E.	—	—	†
1. iii. 1918	Battersea Park	—	—	†
2. iv. 1918	Ruskin Park, Denmark Hill, S.E. ...	—	—	†
2. iv. 1918	Peckham Rye Park, S.E.	—	—	†
29. iv. 1918	Swamp near Southend Pond ...	—	*	
29. iv. 1918	White Foot Lane, Hither Green, S.E.	—	—	†
30. iv. 1918	Hayes, Kent	—	—	†
8. v. 1918	Sydenham Wells Park	—	—	†
10. v. 1918	Streatham Common	—	—	†
10. v. 1918	Pond in adjoining field	—	—	†
14. v. 1918	Mitcham Common	*	—	

* denotes the occurrence of eggs, larvae or pupae.

† denotes conditions apparently favourable, though no immature stages found.

TABLE SHOWING PLACES VISITED, IN ORDER OF DATE (*continued*).

Date	Locality	*A. maculipennis*	*A. bifurcatus*	
14. v. 1918	Tooting Bec Common	—	—	
14. v. 1918	Brockwell Park	—	—	
21. v. 1918	Wimbledon Common	—	—	†
21. v. 1918	Putney Heath	—	—	†
28. v. 1918	Ham Common, Richmond	—	—	†
28. v. 1918	Richmond Park	*	—	
31. v. 1918	Bushey Park	*	—	
1. vi. 1918	Regent's Park	—	—	
2. vi. 1918	Chelsea Physic Garden	—	—	†
4. vi. 1918	Swamp near Southend Pond	—	—	†
5. vi. 1918	Telegraph Hill Park, New Cross, S.E.	—	—	†
8. vi. 1918	Brent Reservoir, near Hendon	—	—	
11. vi. 1918	Gunnersbury Park	—	—	†
12. vi. 1918	Sydenham Wells Park	—	—	†
15. vi. 1918	Hampstead Extension Fields	—	—	
25. vi. 1918	Bloomfield Park, N.W.	—	—	
4. vii. 1918	Finsbury Park	—	—	
8. vii. 1918	Wanstead Park	*	—	
8. vii. 1918	Woodford	*	—	
8. vii. 1918	Higham Park, Epping	—	—	
10. vii. 1918	Ham Common	*	—	
10. vii. 1918	Richmond Park	*	—	
30. vii. 1918	Keston, upper pond	*	—	
1. viii. 1918	Streatham Common	*	—	
1. viii. 1918	Mitcham Common	*	—	
2. viii. 1918	Regent's Park	*	—	
9. viii. 1918	Dulwich Park	—	—	†
9. viii. 1918	Ruskin Park, Denmark Hill, S.E.	—	—	†
9. viii. 1918	Peckham Rye Park	*	—	
10. viii. 1918	Telegraph Hill Park	*	—	
15. viii. 1918	Chelsea Physic Garden	*	—	
15. viii. 1918	Battersea Park	*	—	
26. viii. 1918	Putney Heath	*	—	
26. viii. 1918	Wimbledon Common	—	—	†
2. ix. 1918	Hampstead Extension Fields	*	—	
2. ix. 1918	Golder's Hill Park	—	—	
2. ix. 1918	Hampstead Ponds	—	—	
2. ix. 1918	Highgate Ponds	—	—	
3. ix. 1918	Wanstead Park	*	—	
7. ix. 1918	Regent's Park	*	—	
9. ix. 1918	Greenwich Park	—	—	†
10. ix. 1918	Swamp near Southend Pond	*	*	
11. ix. 1918	Clisson Park, Stoke Newington	—	—	
16. ix. 1918	Staple Inn	—	—	

* denotes the occurrence of eggs, larvae or pupae.

† denotes conditions apparently favourable, though no immature stages found.

DETAILED NOTES ON THE FINDING OF IMMATURE STAGES OF *Culicidae*, TOGETHER WITH PREVIOUS RECORDS OF THE OCCURRENCE OF *Anopheles*, IN LONDON.

1. **Anopheles maculipennis**, M.

Keston, upper pond. Large and shallow, with grassy edges except at one end; weed mainly *Elodea*, but not growing very thickly; numerous small fish, aquatic beetles, Gammarids, and pond-skaters. Visited 30. vii. 1918; wind E., hot and dull. Eggs and young larvae numerous.

Peckham Rye Park. Series of small artificial ponds with flowering water-plants and a considerable quantity of green algae. The water is supplied by a tap which is turned on at intervals of a few weeks, or more frequently during dry weather. Grass at edges kept closely cut; at elevation of 100 ft or less. Visited 2. iv. 1918, when no larvae were found. Examined again 9. viii. 1918; wind W. to N.W., warm and sunny; 7 young larvae found; also 2 larvae of *Culex pipiens.*

Telegraph Hill Park, New Cross. Artificial pond with water-lilies, clumps of rushes, and long grass hanging into water from banks. No larvae found on 5. vi. 1918. On 10. viii. 1918, wind N.W., 4 half-grown larvae.

Southend, near Catford. Reedy swamp on the course of the river Ravensbourne, near Southend Pond. Close to main road, at no great distance from houses; becoming partly dried-up in summer; considerable quantity of duckweed; fish present; water clear. Visited 23. xi. 1917, 29. iv. 1918, and 4. vi. 1918, without finding any larvae. On 10. ix. 1918, strong W. wind, with heavy showers at frequent intervals; level of water raised owing to recent heavy rain; one young larva found and pupae, from one of which a male emerged the following day.

Bexley Heath, S.E. of Woolwich. Larvae in clear water of permanent swampy ground with hoof marks, some duckweed, and no fishes; cattle near at hand, also trees; houses within 100 yards; 27. ix. 1917, bright sunshine, temperature 65°; obs. Mrs A. Macdonald (B. Mus. Map, p. 20).

Erith, E. of Woolwich. (1) Larvae in permanent ditches on Picardy Manor Way—swampy ground, where one ditch spreads out after rain and with muddy pools in the course of the other; water clear, but with some weed in both cases; no fishes seen, a few water-scorpions present; houses within 50 yards; road frequented by children; no cattle near. (2) Larvae in shallow part of a permanent ditch in the marsh, with clear water, some *Spirogyra*, no fishes seen; houses within ¼ mile; cattle

feeding close at hand; no trees; 26. ix. 1917; obs. Mrs A. Macdonald (B. Mus. Map, p. 20).

Battersea Park. At shallow end of the Ladies' Pond, among rushes; 1. iii. 1918, no larvae or pupae found; 15. viii. 1918, wind S.W., hot and sunny, 6 young larvae obtained.

Mitcham Common. Natural pond on golf-course, near L. B. and S. C. Ry; edges very grassy, sloping gradually into water, which contained weed; houses and cattle near, few trees; 14. v. 1918; 5 larvae, very young, with several larvae of *Ochlerotatus dorsalis* and *O. nemorosus* and one larva of *Culex pipiens*; 1. viii. 1918, wind S.E., hot and dull; eggs, larvae and pupae abundant; also all stages of *C. pipiens* swarming.

Putney Heath. Pond very much overgrown with rushes, leading into ditch at each end; at side of main road; cattle and trees near, fishes seen. 21. v. 1918, no larvae found. 26. viii. 1918, wind W., showery, fine at intervals, warm; mature larvae and pupae numerous.

Richmond. (1) *Ham Common.* Broad ditch outside Ham Gate, bordered with rather thickly growing shrubs; water containing a quantity of dead leaves; trees and cattle near, houses at some distance. 28. v. 1918, no stages found. 10. vii. 1918, larvae and pupae numerous.

(2) *Richmond Park.* Three ponds lying close together at a short distance from Ham Gate; two of these were practically similar in character, having the margins somewhat grassy and with short stiff sedge, and containing much *Ranunculus.* The third was very shallow and grassy and when visited on 13. ix. 1918 was almost dry. On 28. ix. 1917, numerous mature and nearly full-grown larvae and several pupae were obtained; on 2. x. 1917, larvae and pupae were rare, but occurred in all three ponds; on 28. v. 1918, 6 young and 2 more developed larvae were obtained from the first two ponds, while none were found in the third; 10. vii. 1918, all stages numerous in the first two ponds and a few in the third; 13. ix. 1918, 3 mature and 4 younger larvae from the first two ponds only.

The Rookery, Streatham Common. Small ornamental ponds with water-lilies and filamentous algae, connected by deeper ditches overhung with shrubs; houses at a considerable distance. On 10. v. 1918, no larvae found. 1. viii. 1918, wind E. to S.E., hot and cloudy; from one of the deeper ditches were obtained 4 young larvae; the water had been changed about two weeks previous to examination and was partly covered with duckweed.

Bushey Park. In broad stream connected with two ponds lying on the Hampton side of the Diana Fountain; edges with coarse grass, forget-

me-not, brook-lime; tadpoles and dragonfly nymphs and pupae abundant. 31. v. 1918, wind S.E., hot and sunny; 2 mature larvae found.

Chelsea Physic Garden. Several artificial ponds with algae, *Elodea*, water-lilies or rushes; 27. xi. 1917 and 2. vi. 1918, no larvae found; 15. viii. 1918, in one pond with many rushes were found 8 larvae (imagines reared).

Hampstead Extension Fields. Muddy swamp, overgrown with reeds, enlarging at one end into a shallow pond with filamentous algae and flowering plants; trees near, houses at no great distance. 15. vi. 1918, no larvae found. 2. ix. 1918, from pond were taken two larvae and one pupa, also *C. pipiens*; from swamp, *Culex pipiens* and 2 larvae of *Ochlerotatus nemorosus*.

Regent's Park. Artificial stream in the grounds of Bedford College. Stream arranged in a series of terraces; water had not been turned on for some months, but rain had collected to varying depths; water contained fallen leaves, while long grass hung in from edges. 22. xi. 1917 and 1. vi. 1918, no larvae found. 2. viii. 1918, water at a depth of about six inches, wind S.E. to S.; numerous larvae, mostly well-developed, a few young forms; swarms of larvae and pupae of *C. pipiens* present. 7. ix. 1918, 4 young larvae, also *C. pipiens*, though less abundant than in August.

Wanstead Park. Three large ponds, *A*, *B*, and *C*. *A*, nearest the main road, is shallow, with clumps of rushes at either end, banks not grassy; *B* has concrete edges, no plants, and is used for boating; while *C* is shallow, especially at one end where there are water-plants, and is surrounded by trees. 8. vii. 1918, numerous larvae among the rushes in *A*; 3. ix. 1918, 2 full-grown larvae from shallow end of *C*.

Woodford. Small pond at the side of the main road immediately beyond the termination of the electric tramway. Water dirty, containing much weed, mainly *Ranunculus*, debris, filamentous algae and dead leaves. 8. vii. 1918, eggs, larvae and pupae abundant; a second larger pond, further from the road, deeper and with less weed, yielded a few larvae.

London, Albert Dock. Larvae, 1901 (B. Mus. Map, p. 14).

2. **Anopheles bifurcatus**, L.

Southend, Catford. Swamp on the course of the river Ravensbourne (see under *A. maculipennis*). 23. xi. 1917, 19. ix. 1917 and 26. ix. 1917, numerous larvae found, also larvae of *Theobaldia morsitans*; 29. iv. 1918, 2 mature larvae and several pupae; 4. vi. 1918, larvae and pupae of

Culex pipiens only; 10. ix. 1918, 2 half-grown larvae, also *A. maculipennis.*

Richmond. (1) At 50–100 ft (*a*) a few larvae in one (middle) pond of Pen Ponds in Richmond Park; absent in upper pond, though conditions are similar; margins grassy; short rushes; (*b*) larvae fairly numerous in a similar pond in park near Ham Gate; (*c*) many pupae and a few larvae in a little grass-bordered stream near Roehampton Gate. Obs. L. Cobbett, 18. x. 1900 (B. Mus. Map, p. 46).

3. **Anopheles plumbeus,** Steph.

Epping Forest. In root-holes in trees. Obs. A. Bacot (B. Mus. Map, p. 49).

4. **Anopheles** sp.

Mitcham Common. (1) 5 larvae, with *Corethra* and *Chironomus* larvae, in sedgy pool behind monument on road between Mitcham and Croydon, 26. ix. 1917; det. Prof. H. M. Lefroy. (2) 8 larvae from next pool in same place, x. 1917; subsequent weekly visits all through x. and xi. failed to produce any larvae. Obs. Miss L. E. Cheesman (B. Mus. Map, p. 57).

Wimbledon Common. 3 larvae in a ditch bordering the high road, 15. ix. 1917; obs. Miss L. E. Cheesman (B. Mus. Map, p. 57).

5. **Culex pipiens,** L.

Golder's Hill Park	2. ix. 1918	
Mitcham Common	26. viii. 1918	Swarms of eggs, larvae, and pupae, with those of *A. maculipennis*
Peckham Rye Park	9. viii. 1918	Two larvae, with larvae of *A. maculipennis*
Regent's Park	2. viii. 1918	Eggs, larvae and pupae abundant, with larvae of *A. maculipennis*
Richmond	28. v. 1918	A few larvae in pond near Ham Gate
Southend, Catford	4. vi. 1918	Larvae and pupae
Streatham Common, in field adjoining the Rookery	1. viii. 1918	
Sydenham Wells Park	12. vi. 1918	One pupa
Wimbledon Common	26. viii. 1918	A few larvae and pupae in pond near main road; larvae and pupae in pond near windmill

6. **Theobaldia morsitans,** Theo.

Southend, Catford	19. x. 1917–23. xi. 1917	Larvae numerous, with those of *A. bifurcatus*
White Foot Lane, Hither Green	29. iv. 1918	One larva, with larva of *O. dorsalis*

7. **Ochlerotatus dorsalis,** Mg.

Mitcham Common	14. v. 1918	Larvae and pupae numerous, with larvae of *A. maculipennis* and *O. nemorosus*
White Foot Lane, Hither Green	29. iv. 1918	One larva, with larva of *Th. morsitans*
Wimbledon Common	21. v. 1918	Two larvae in ditch near main road

8. **Ochlerotatus nemorosus.**

Hampstead Extension Fields ...	2. ix. 1918	Two larvae in reedy swamp
Mitcham Common	14. v. 1918	Larvae and pupae, with larvae of *A. maculipennis* and *O. dorsalis*
Wimbledon Common	21. v. 1918	Several pupae in ditch, with larvae of *O. dorsalis*

IMPERIAL COLLEGE OF SCIENCE,
LONDON.

THE DISTRIBUTION OF PARASITE-INFECTED FISH.

By H. CHAS. WILLIAMSON, M.A., D.Sc.

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Among the fish landed at Aberdeen there are two notable classes that are infected by parasites, and it appears that they can be traced to two definite areas of the sea. I refer to what are known as "spotted haddocks," and "worm-infested codlings."

The spotted haddock is a fish of plump condition, in the muscles of

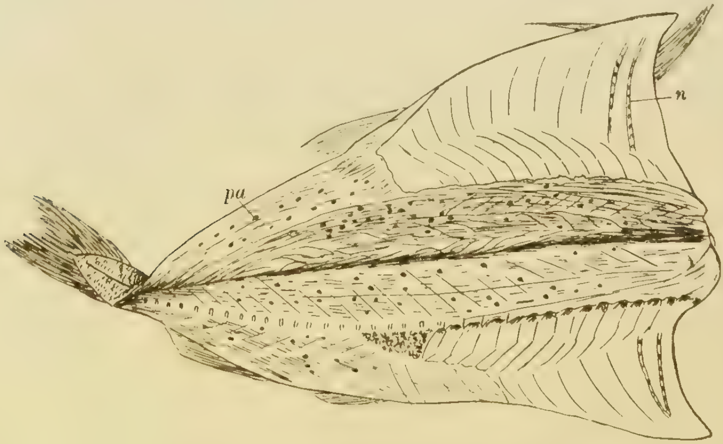

Fig. 1.

which are distributed the cysts of a peculiar parasite which has been named *Dokus adus* Fig. 1, *pa*. A somewhat similar organism was found by Hofer in diseased trout. The infected trout became lethargic and swam about intermittently in a staggering fashion. Hofer regarded the parasite as a sporozoan. Pleyn and Mulsow however found a fungoid-like budding in cultures of the cysts, and they came to the conclusion that it was a fungus. They named it *Ichthyophonus hoferi*. The parasite was

found in the liver, kidney, heart, muscles, and sometimes the brain of the trout. The staggering movements were not however always present.

Johnstone has described a fungus, apparently nearly related to the preceding; it occurred in plaice kept in the hatchery pond on the Isle of Man. Infected fish swam about languidly, but showed neither evidence of giddiness, nor lack of co-ordination in their movements.

Externally the spotted haddock is often firm and hard: but when it is split for curing, the flesh emits an unpleasant smell, which is to some extent reminiscent of creasote. The smell may not be noticed in the fresh condition, but it becomes evident after the fish has been smoked. If the cured fish are kept hanging a day after smoking, before they are packed, the odour seems to be accentuated. The smell may be detected in the smoke kiln. Such fish are said to have a sour taste: the flesh when smoked has sometimes a greenish tint and, when observed, they are rejected.

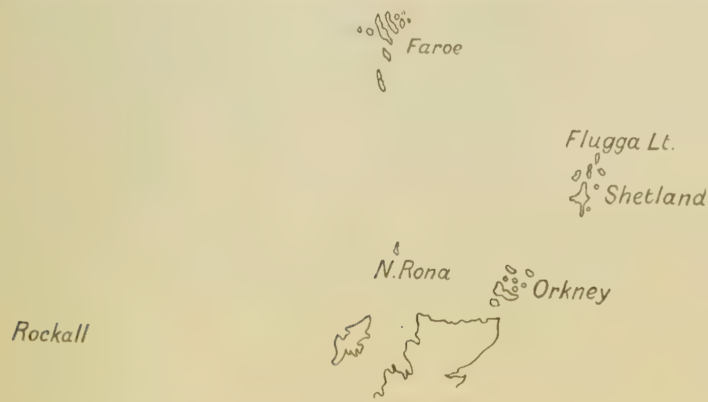

Fig. 2.

The main weight of the evidence at present available points to the conclusion that the spotted haddocks are not found among the North Sea fish landed at Aberdeen, although in the opinion of some observers they are so. There is however general agreement that they occur frequently among the haddocks caught at Shetland, both line- and trawl-caught. The West of Orkney also is given as a locality of origin. Difference of opinion exists as to whether they come from the West of Scotland. Infected fish, both large and small, are found all the year round, but more commonly in warm weather.

These haddocks are generally also infected with trematode cysts in the anterior spinal nerves which are visible on the inside surface of the abdomen (*n.* Fig. 1). Some of the haddocks caught near Aberdeen have these trematode parasites, an infection which is recorded by Lebour as common.

The spotted haddock is not known to occur in either the Iceland or Faroe fisheries. The Faroe haddock appears to be on the whole richer in flesh than the Shetland or North Sea fish, and it does not lose condition after spawning, so much, or for so long a period as the fish from the two other regions.

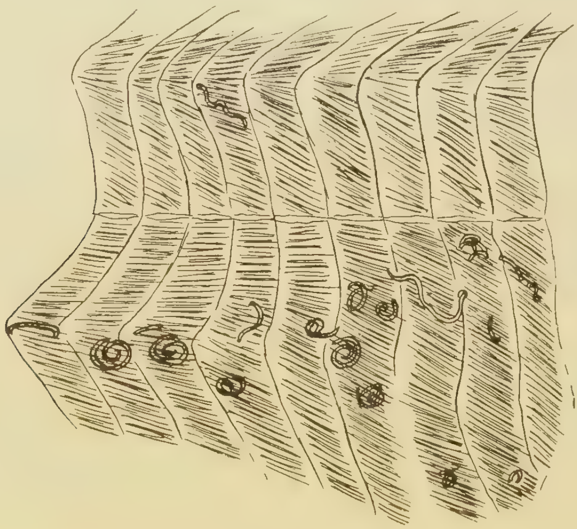

Fig. 3.

The spotted haddock was, I am informed, unknown in Aberdeen seventeen years ago, and one gentleman who cured haddocks on the west coast of Shetland (Walls, Scalloway) twenty-five years ago did not know them. It is said however, that defective haddocks, possibly spotted, had been sometimes taken twenty-five years ago in June in nets which had sunk to the bottom with weight of herrings, between Ve Skerries and Blue Mull Sound.

The affected codlings exhibit coiled nematodes scattered singly through the muscles: they are taken at Faroe, Rona and Rockall: especially in the first-mentioned fishing region. At present it is believed

they do not occur among North Sea fish. The infection at Faroe appears to be seasonal, *e.g.* in summer.

Fig. 3 exhibits a portion of the flesh of an infected codling. It was obtained from an Aberdeen fish-yard. The place of origin of the fish was not ascertained. Fig. 4 shows a matted group of nematodes (*N*) inside the abdominal cavity of the same fish: the worms are attached to the peritoneum (*pt*).

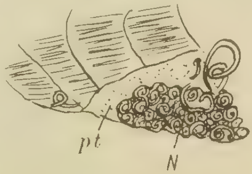

Fig. 4.

The nematodes do not seem to injure the muscle: they are immature and may be about one inch in length. They resemble in some respects larval stages of *Ascaris decipiens* described by Linstow. Larval and adult specimens of this worm were found in profusion in the alimentary tract of two species of seal.

The muscle worm is a very resistant form: it survives the preparation of the fish into the smoked fillet. During that process it is in brine pickle for half-an-hour, and afterwards smoked for from three-quarters of an hour to two hours. A fillet prepared in this way was kept for thirteen days: at the end of the period it smelled offensively. Live worms were dissected out at intervals from the first to the thirteenth day. In a second case the fillet was exposed to the brine and smoke for double the usual time. Thereafter two of four nematodes which were extracted were found to be alive. Nine days later a number of worms were taken out, but all were apparently dead. In a third experiment the cod fillet was dried in the open air: no salt was used. Three months later the fish was very hard. When some of the worms from it were put into sea water they swelled up and became plump. A little movement was detected in one or two cases, but there was no clear evidence that any individual was actually alive.

The worm-infection of the muscle is not restricted to the codling: it occurs in tusk, saith, and I believe also in haddock. The distribution of the affected members of these species is less clear.

It is interesting to note that the spotted haddock occurs at Shetland, but is absent from Faroe; the worm-infected codling exhibits the reverse distribution. A locality or environment may thus have a favourable or unfavourable selective influence on its fish population. The two regions form the margins of the 150 miles wide channel through which the warmer and salter Atlantic current pours into the Norwegian Sea. Trawling is limited on each side to a zone approximately 30 miles broad: the channel has towards its middle a depth of over 600 fathoms. The

deepest part of the channel is stated by Helland-Hansen to be covered nearly always with water of a temperature below 32° F. This water is an offshoot from the bottom water of the Norwegian Sea. It has been found that the bottom water runs nearer to the surface in the southern than in the northern part. The depth of the Atlantic current may be from 2 to 300 fathoms. Owing to the influence of the earth's rotation it is, according to Petersson, bent to the east and it sends a branch round Orkney and Shetland over the northern North Sea plateau into the depths of the Norwegian channel as far as to the Skager Rak. The trawl fishery is carried on in this water of Atlantic origin over the whole northern North Sea plateau (deeper than 43 fathoms), as far as the Dogger Bank, and over the western and southern slope of the Norwegian channel (Great Fisher Bank, Jutland Bank, etc.).

Whether the Atlantic current has any special influence with regard to the parasite-infection of the fish is an obscure question, as is also the question whether the fish caught at Shetland for example have been born in some other region. Schmidt deals with the distribution of the fry of the cod, haddock, etc. He says that the pelagic fry (*i.e.* the early stages which have not yet taken up life on the bottom) of the cod produced on the coastal banks at Iceland cannot be carried away from that island to other coasts by currents. It is said however that Iceland cod occur among the fish caught at Faroe and landed at Aberdeen. It would appear also that the fry do not leave the Faroe region to any important extent, with the exception perhaps of some migration from the banks south of the islands. The North and Norwegian Seas receive from the Atlantic a large import of haddock, ling, etc. in the pelagic stage.

Until more extended investigations are made, it may be tentatively concluded that the infected haddocks and cod are restricted in their distribution, and one would be inclined therefore to look for the cause in the local environmental factors, but the problem is a very wide and intricate one.

OBSERVATIONS ON THE HABITS OF CERTAIN FLIES, ESPECIALLY OF THOSE BREEDING IN MANURE.

By J. E. M. MELLOR, B.A.

(With 6 figures and 4 Charts.)

CONTENTS.

PREFACE.

SINCE the relation of flies to certain diseases—and the possibility of that relation being of importance—was first appreciated, much attention has been directed to their control, and their habits and life histories have

been widely studied. The problem of fly-control may be dealt with by three methods: (1) destruction of the adult; (2) destruction of the immature stages; (3) perfect and thorough sanitation.

Flies are a corollary of insanitary conditions. "No dirt, no flies." In those parts of modern cities, in which strict sanitation is observed, flies are, probably, not of great importance; but little hope can be entertained of freeing the poorer parts of our own towns and cities, much less those of Eastern countries, from the danger of their presence until legislation and education are much improved.

Method 3, therefore, entails, not only legislation but the alteration, in many cases, of ancient habits, and so is not likely to be applied immediately, still less universally. Method 1, as will be shown later, can never be either thorough or permanent.

Under these circumstances the importance of adopting the second method cannot be too strongly advocated. There are, however, occasions when circumstances necessitate the use of the first method: for instance, when either it is not possible to obtain authority to control the breeding places; or they are too large to be dealt with by the means at hand.

Since the house-fly (*Musca domestica*) has such a wide geographical distribution, it is probable that its life history is variously modified in different parts of the world. This modification will probably depend on climatic conditions—especially those of sunshine and temperature—and the presence or absence of insect and fungoid enemies. Portchinsky (1913) writes that in July 1911 he found very large numbers of *M. domestica* in and about the houses and stables of newly founded farms, on the dry arid steppes, in the southern part of the Government of Stavropol (N. Caucasus); that there were no *Hydrotaea*, *Myospila meditabunda*, or *Polyetes albolineata*, and that *Muscina stabulans* was rarely found on these farms; and that in the open country away from the houses *M. domestica* flourished in enormous numbers in the total absence of their chief enemies.

In England and countries with a similar climate, the year may be divided into two seasons as regards fly control—namely, May to the beginning of November and from November to May (Graham-Smith (1916) chart 4)[1]. In summer it seems hopeless to attempt to deal thoroughly

[1] This refers to flies generally. As regards *Musca domestica* the period during which the adults are most numerous lasts from July to October. The remainder of the year presents the weakest part of the life-history of the house-fly. If breeding places were rigorously attacked particularly at this season, the numbers emerging between June and October would be greatly diminished.

with the fly pest by method 1—destruction of the adult. Howard's figures (1912) give an unnecessarily alarming idea of the number of descendants from a single pair of flies. Graham-Smith (1916) showed that the descendants of each female blowfly during the season numbered 130 individuals instead of 1012 millions.

In Howard's calculations no account was taken of natural dangers, such as lack of food, the ravages of carnivorous larvae, hymenopterous parasites and inclement weather.

On the other hand, the escape of but a few females may be sufficient to ensure the continuance of the multitudes of flies. Moreover, owing to the extremely short preoviposition[1] period, the chance of killing the majority of females before they oviposit seems to be very meagre.

Griffiths (1908), working in England, found this period to be 10 days in the case of *M. domestica*[2]; Hewitt (1910), also working in England, found it to be 14 days. Bishop, Dove and Parman (1915), working in Dallas, Texas, found the period to be only 4 days in summer and not less than 10 days in spring and autumn.

At present all that can be expected of this method is temporary local relief, such as the clearance of hospital wards, of individual rooms, etc.—and this only when fresh invaders are prevented from entering by placing muslin or wire screens over windows and doors. Such screens tend to diminish the freshness of any breeze entering, and so cause some discomfort in hot climates. Whilst trying to reduce the swarms of flies at a military hospital in Serbia, in 1915, I had to resort to method 1, owing to the lack of sufficient means to attack the breeding places successfully. I found it difficult to get some men to refrain from tearing the muslin placed over the ward windows, and impossible to get muslin-covered swing-doors, especially made for the kitchen, kept closed. The cooks, who were peasants, complained that the muslin kept out the breeze, and, not realising the danger to which they were exposing the hospital, preferred flies and breeze to no flies and less air; and the Commandant was not sufficiently alive to such matters to be aware of the advisability of enforcing the necessary regulations. Those wards, in which the necessary precautions were observed daily, were kept almost free from flies to the great relief of the patients.

[1] The period between escape from the puparium and oviposition, during which the fly becomes sexually mature and copulation takes place.

[2] *Musca domestica* forms at least 90 per cent. of the flies frequenting houses and is, therefore, the most dangerous. (Hermes, 1911, p. 521, quoted by Graham-Smith, 1914, p. 79.)

The adoption of the second method cuts nearer the root of the evil, and where suitable sanitary arrangements or efficient larvicides are available much more effective work can be done even in summer.

But, provided that suitable methods are forthcoming, winter would probably be the best season in which to attack the problem. The numbers of flies have then been reduced by natural deaths, the ravages of *Empusa muscae* and of Hymenopterous parasites, and carnivorous larvae are still taking their toll.

Portchinsky (1913) considers that the larvae of *M. stabulans* and of *H. dentipes* wreak untold havoc amongst the larvae of *M. domestica*. Graham-Smith (1916) thinks that he failed to bring the larvae of *M. domestica* through the winter of 1914–15 because those, which survived heavy rain, were destroyed by the larvae of *H. dentipes*, the progeny of a single female, which accidentally gained access to the breeding cage.

In view of supplementing the work of Nature by artificial means, it is important to discover where and in what stage, or stages, the insect passes the winter.

The writers on hibernation may be divided into four groups: Group I which holds the opinion that *M. domestica* passes the winter in the adult stage; Group II which considers that, though the adults which survive the winter are the most important factor in perpetuating the species, it is possible, but not sufficiently proved, that the pupal stage also survives; Group III which recognises that some adults do undoubtedly over-winter in suitable situations, but consider that the immature stages are of most importance in the continuance of the species in the following year; and, finally, Group IV which believes that *M. domestica* hibernates only in the pupal stage.

In Group I appear Newstead (1909) and Hewitt (1914 and 1915). The former (1907) wrote that, while the adults were found in the dwellings of man throughout the winter and in early spring, "Whether they pass the winter entirely in this stage one has not been able to ascertain. It is highly probable, however, that some of the pupae may remain over the winter and hatch in the following spring." But as a result of a single experiment, carried out in October 1907, the same observer (1909) concluded that the house-fly did not pass the winter in the larval or pupal stages—"so that as far as one can trace at the present moment, the only stage in the life cycle, which is found during the winter months is that of the adult flies." Hewitt (1912) considers that "it is not unlikely that larvae of *Fannia canicularis*, which developed late in the season, pass

the winter in the pupal state, as is the case with certain *Anthomyidae*"; and (1914, p. 107) has kept *Stomoxys calcitrans* over winter as pupae: but thinks that *M. domestica* only winters in the adult stage. He points out that the abdomens of those caught in winter are full of fat and that the alimentary canal is shrivelled up. He writes (1915) that he has always held the view suggested by Copeman and Austen (1914) "that the relative lateness of the season at which house-flies annually become abundant may be due to the smallness of the numbers of individuals that in an active, or inactive condition, survive the winter in houses or buildings."

Graham-Smith (1914), summarising the opinions on this subject up to 1914, wrote that "flies may be found active throughout the winter in relatively high temperature, *e.g.* warmed houses, kitchens, bake-houses, and in the presence of sufficient food material may even continue to breed, but little is known as to their method of passing the winter under natural conditions. Most observers, however, seem to think that the winter is passed in the adult condition."

In Group II may be placed Jepson (1909) and Copeman (1913). The former considered that autumn flies were hardier than summer ones, and found them in kitchens at temperatures of 65° F.–80° F. "quite as active as in summer," and persuaded them to breed and oviposit on soaked bread. He also found sluggish specimens behind books on a bookshelf in December and January; but failed to bring 200 pupae alive through the winter.

The latter wrote "as to whether flies can persist through the winter in other than the adult form practically nothing is known; but as the eggs and larval stages, at any rate, appear to be less resistant to the effects of reduction of temperature than the fly itself, it is probable that the progeny of the later broods, for the most part, never arrive at maturity, both eggs and larvae perishing in their breeding places," and that, though it would seem that the pupae might stand the winter better than the earlier stages, he knew of no instance of their having been successfully wintered. The examination of the thatch of a hen house in March 1913, of two attics in March and May respectively, and of 25 hay and corn stacks in April showed the complete absence of *M. domestica* in those places. And Austen, who identified the specimens collected, remarked, of those which were found, that the predominance of females to males was so slight as to be practically negligible; and was not what one would expect from hibernating flies.

In Group III appear Williston (1908), Howard (1911 and 1912),

Copeman and Austen (1914), Graham-Smith (1914 and 1916), Bishopp, Dove and Parman (1915) and Dove (1916)[1].

As early as 1908 Williston stated that the winter was passed in the puparia, but that in secluded spots mature flies will sometimes survive the winter. He does not, however, quote any experiments to qualify these statements.

Howard (1911) wrote that *M. domestica* hibernates "in the puparium condition in manure or at the surface of the ground under a manure heap. It also hibernates as adult, hiding in crevices": and in 1912 "Adult flies undoubtedly linger in warmed houses throughout the winter, but that enough of them remain in active condition in such locations to perpetuate the species and start the rapidly multiplying generations of the following summer seems doubtful"; but thinks it very probable that the

[1] Since the Preface and account of my observations on the Wintering habits of flies were written, two papers have been read, the authors of which claim to have successfully bred *M. domestica* from over-wintered pupae: and, further, certain of their observations are of interest in connection with my Burial Experiment, pp. 80–84.

I. Kisliuk (1917) found that (1) flies which are not kept cold enough to become inactive (see Dove, 1916) will either oviposit, if the temperature is high enough, or die comparatively soon; (2) under natural conditions neither eggs nor larvae are to be found alive in normally preferred situations, though the latter may probably be found in early winter (these observations were made in America, but the latter one does not tally with those made by Graham-Smith (1916), McDonnell and Eastwood (1917) and my own (1916–1917) described in this paper, when larvae of various flies were found throughout the winter). (3) From pupae collected from natural situations on February 26, a few flies emerged on March 10th–12th, though 91 per cent. were parasitised, which, he considers, indicates that *M. domestica* hibernates as a pupa. And from breeding experiments he concluded that the following species hibernate in immature stages: *L. sericata, L. caesar, L. sylvarum, Phormia regina, C. erythrocephala, C. vomitoria, Cynomyia cadaverina*, and *P. rudis*, which also does so as an adult.

II. McDonnell and Eastwood (1917) made the following observations: (1) "On March 3rd, 1917, living larvae were found in a heap of old manure at a depth of 3 *feet*. This heap had not been touched since October 1916 and was overgrown with grass and weeds. On March 20th larvae were found at a depth of 2 feet in a mixture of dry earth and human excreta, which had been made in September 1916, and was covered with a 6 inch layer of earth". (2) "Larvae found at a depth of 2 feet in a manure heap were apparently dead, but revived, when exposed to heat. Evidently they were hibernating." (3) Pupae, which had migrated from the manure in the larval state, were found 2 feet from their respective dumps at a depth of about an inch below the surface. (4) The larvae nearly all pupated within 24 hours of removal from the manure heap, but one or two were still persisting as larvae on April 14th. (5) During first week in April pupae developed into flies—*F. canicularis* and *M. domestica*.

From these observations the authors conclude that, as living fly larvae were found, in March, in earth and human excrement made six months previously, reliance cannot always be placed in the method of disposal in shallow trench latrines as a preventative of fly breeding.

adults do remain dormant in cold and sheltered situations. He considers that insufficient attention has been paid to the accurate identification of species found. That this may often be the case is shown by the observations of Copeman (1913) already quoted.

In 1914 Copeman and Austen, as a result of examination of 58 consignments of flies, sent from all parts of England, only found *M. domestica* to appear 12 times, of which 9 were observations of single specimens, one of the remains of 11 dead flies (Appendix, Serial No. 4), one of several specimens reported in a baker's shop (App. Ser. No. 17), and one of a single specimen reported to have emerged from a stable manure heap near the house, from which it was thought possible to obtain several flies on any mild day throughout the winter[1].

From the results of this investigation the writers consider that the customary explanation of the perpetuation of the house-fly by over-wintered adults had been fairly tested and found to fail. They therefore suggested looking for pupae during the winter in places where adults were known to have been abundant during the previous summer and autumn.

In an earlier part of the paper they suggest that the relative lateness of the season, in which the house-fly becomes abundant, may be due to the fact that only small numbers of adults overwinter, and that therefore it requires time for the numbers to increase to any importance. They remark, however, that "there is as yet nothing in the shape of proof that the female house-flies, found alive at the end of winter, actually survive until oviposition takes place." As regards this, Graham-Smith (1914) has shown that the increase in numbers of flies in autumn is closely connected with the temperature recorded by the 2 foot ground thermometer, and (1916) that the sudden disappearance towards winter is due to the non-emergence of flies from pupae, owing to the temperature falling below the critical point necessary for the emergence of *M. domestica*.

Graham-Smith (1914), though he has not actually found the pupae of *M. domestica* during the winter, records observing numerous blow-flies (*Calliphora erythrocephala*), which seemed to have freshly emerged, on sunny days at the end of February 1914, in sheltered gravel pits far from houses. In April and May 1913 he bred *Sarcophaga melanura* and *Anthomyia radicum* from samples of dog's faeces, collected in the autumn of the previous year. And, moreover, on March 28th, 1914,

[1] Possibly the heap and its environs contained many pupae from which these flies emerged at intervals during winter, and the remainder in summer.

obtained pupae of different species from the earth near manure heaps and hatched flies from them. He summarises that the "evidence at present available seems to indicate that few house-flies hibernate as adults, and from observations on other species of flies, the writer is inclined to believe that the winter is passed in the pupal stage": but "the pupae of *M. domestica* have not yet been found." The same author (1916) found that of a few blow-flies, which emerged from pupae from time to time throughout the winter, some lived for several weeks and survived heavy rains, snow, frost, cold wind and gales; but, on the other hand, proved by experiment that the following flies hibernate as pupae, or, less commonly, as larvae in the earth, under shelter 2–3 inches below the surface of the ground or under shelter on the surface:

Calliphora erythrocephala, Mg.
Fannia manicata, Mg.
Fannia canicularis, L.
Fannia scalaris, F.
Anthomyia radicum, L.
Tephrochlamys canescens.
Blepharoptera serrata, L.
Scatophaga stercoraria, L.
Dryomyza flaveola, F.
Calliphora vomitoria, L.
Muscina stabulans, Fall.
Muscina pabulorum, Fall.
Nemopoda cylindrica, F.
Piophila vulgaris.
Hydrotaea dentipes, F.
Sarcophaga melanura, Mg.
Sarcophaga carnaria, L.
Stomoxys calcitrans, L.
Mydaea lucorum, Fall.
Lucilia caesar, L.
Lucilia sericata, Mg.
Phaonia erratica, Fall.
Ophyra leucostoma, W.
Polietes lardaria, F.

and considers that "a few individuals, insignificant in number compared with those passing the winter as pupae, hibernate in the adult condition" but that, in perpetuation of the species, these are of little account; yet "the wintering habits of *M. domestica* are still obscure."

Bishopp, Dove and Parman (1915) at Dallas and Uvalde, Texas, found that the winter could be passed in the immature stages, and, moreover, none of the adults under observation survived the winter. Their results indicate that flies kept at a temperature not low enough to render them inactive, either oviposited very soon or died[1]. They think the chances of adults finding shelter from destruction by cold very small indeed, and that, although some adults may hibernate thus, the species is dependent on those individuals which winter in the immature stages or continue to breed throughout it.

Dove (1916) found that larvae and pupae of *M. domestica* overwintered as such at Dallas and at Uvalde, Texas, and emerged on mild days in

[1] Vide Dove's (1916) suggestion, quoted later, that *E. muscae* chiefly attacks mature females which have not oviposited.

winter at temperatures below 45°–55° F. When fresh manure was not added to the experimental heap emergence ceased owing to the fall of temperature, which had been kept up by fermentation. But he thinks that pupae, which were located near the surface of the soil, received either enough heat for emergence or were probably killed by cold. He found that flies tend to seek a temperature of 60° F., but that, given that they had sufficient food, their only chance of living for any length of time was to remain dormant. He suggests, as the result of a single experiment, that *Empusa muscae* developes principally in sexually matured and fertilised females, which do not oviposit on account of cold or lack of suitable media.

Finally Group IV is represented by Skinner (1913), who observed fresh specimens of *M. domestica* entering his laboratory window on March 13th, 1913, and states that, until disproved, he will answer the question "Do flies hibernate?" thus, "house-flies pass the winter in the pupal stage and in no other way."

At the suggestion of Dr Graham-Smith, a careful examination of manure heaps and their vicinities and other likely places, for larvae and pupae, was started in January 1916, and was continued during the winter 1916–1917. During the summer of 1916 and the early portion of that of 1917 various experiments and observations were made, a full account of which will be found in the Cambridge University Library (*Research Student's Dissertation*, 166). In this paper a short account only is given of observations made on overwintering and summer distribution of flies; and of three of the experiments, carried out during the summer, to investigate (1) the temperature of horse manure heaps; (2) the effect of applications of Creosote Oil Mixture and the best method to apply it; and (3) to discover whether the burial of material infested by the fly larvae, at a depth of four feet, would prevent the developement or escape of the adult, where the larvae would pupate under these conditions and to what height the adults were capable of climbing in various soils, loose or tightly packed.

This work was carried out on a grant from the Medical Research Committee and under the direction of Dr G. S. Graham-Smith, to whom my sincere thanks are due for his ever ready help and encouragement throughout the investigation. My thanks are also due to Mr Forman for much help and suggestion in the designing of apparatus in Series 3 (facing pp. 69, Figs. 1, 2, and 70, Figs. 3, 4), and to Mr C. G. Lamb and to Dr Keilin for their kindly help and sympathy and for identifying certain of the flies caught or bred during the experiments.

Winter Observations.

The winter search for flies in immature stages in natural situations was started in January 1916 and was continued during the whole winter of 1916–1917. Between January 27th and April 20th, 1916, 14, and between September 22nd, 1916, and April 23rd, 1917, 31 likely places were examined, 45 in all. Of these, 13 were horse manure heaps or near horse manure; 12 were in or near cow manure heaps; 4 in or near horse and cow manure mixed; 6 in or near pig manure; 1 in fowl manure; 3 in ash middens; 1 in a refuse pit; 1 a town road-scrapings tip; 1 in ground in corner of a slaughter-house yard; 1 in soil under a spot over which human faeces had been exposed during the previous autumn; 1 in soil under a spot over which cow manure had been similarly exposed; and 1 in the nests of House-martins and Pigeons in an old mill.

Thirty-seven of these places were in Cambridgeshire, 7 in Norfolk and 1 in Argyll: 29 were in the country and 16 in the town of Cambridge.

Besides the larvae and pupae collected from these sources, some were taken from cow manure and others from the neighbourhood of horse manure, which had been exposed during the previous autumn in the course of other experiments.

The only observations of this nature heretofore recorded are those of Graham-Smith (1914 and 1916) and of Bishopp, Dove and Parman (1915). The former examined soil near manure heaps in Cambridgeshire, England, on March 28th, 1914, and, in damp soil under a hedge near one heap, at a depth of about 6 inches, found several pupae of *Calliphora erythrocephala* and one of *Ophyra leucostoma*; and "in soil close to another heap, which was situated in an open field" a number of pupae from which *Anthomyid* flies eventually emerged.

Bishopp, Dove and Parman, working at Dallas and Uvalde, Texas, found considerable numbers of House-fly larvae in chicken manure in poultry houses in mid-winter, where they consider the conditions "especially favourable for the immature stages to pass the winter, as the manure generated very little heat, yet being within the chicken house the insects are not subject to excessive cold." They also found great numbers of larvae in Livery barns, which furnished similar conditions, "in cracks in the floors and in corners of the stalls."

Methods employed.

The larvae were always taken to the laboratory in some receptacle supplied with holes to admit a plentiful supply of air, placed in jam jars

(size 1–2 lb.) and given a little of the manure in which they had been found—when this material was very moist a little dry earth was added to tempt them to pupate.

Throughout the investigation it was found very difficult to overcome the efforts of the larvae to migrate from the jars, in which they were confined. A glass filter funnel was dropped into the mouth of each jar, thus at once closing it and admitting air. However, in spite of this, some larvae did either find a spot where some irregularity in the rim of the jar permitted them to squeeze between it and the filter funnel, or actually made their way up the inside of the funnel. Very few managed this gymnastical feat, and the first path of escape was effectually blocked later by a layer of plasticine.

The temperatures recorded were taken with an ordinary centigrade thermometer placed in a large glass tube, held in position by a piece of cork, and surrounded at the bulb end by paraffin wax. The bulb end of the glass tube was closed with a cork, the opposite end being left open. This device, though prolonging the time necessary to leave the thermometer in the manure, enabled the temperature to be read before the mercury fell on the withdrawal of the tube from the heap.

A table giving the species found in the larval or pupal stages, from which adults were actually bred, is given; those which are additional to Graham-Smith's (1916) list being marked; the kind of manure in which each species was found, the dates of findings and the sexes, which emerged, are also shown (pp. 64, 65).

Summary of observations.

1. Thirty-nine species were actually bred from larvae or pupae found in natural situations during the winter; 31 of these are additional to Graham-Smith's (1916) list.

2. Larvae of *Dolichopodidae* were found in soil near cow manure, and were identified by Dr D. Keilin, but were not successfully reared.

3. Pupae of *Musca domestica* were found during winter in horse manure but the adult was not reared.

4. It is possible that larvae and pupae of other species were found, though they were not successfully reared.

5. The distribution of dipterous larvae in a manure heap in winter, in the Eastern Counties, is extremely local. Similar observations were made at Dallas and at Uvalde, Texas, by Bishopp, Dove and Parman (1915), but they do not state whether their observations referred to both summer and winter, or to either period in particular.

TABLE I.

List of Flies actually bred from Larvae and Pupae collected during the Winters 1915–1916 *and* 1916–1917.

Flies marked with * are additional to Graham-Smith's (1916) list.

	Fly	Horse Manure	Cow Manure	Horse and Cow Manure	Pig Manure	Fowl Manure	Dates on which found	Sexes emerged	Dates of Emergence
1	*Sciara* sp.*	LLL (?)	—	—	—	—	19. iii; 6. iv. 17	—	25–30. iv. 17
2	*Scatopse notata**	L	—	—	—	—	2. iii. 17	—	22. iv. 17
	,, ,,	—	—	—	LL	LLPP	27. iii.; 6. iv. 17	8 ♂ 5 ♀	26. iv. & 2. v. 17
3	,, sp.*†	—	—	—	—	LL	—	—	—
4	*Dilophus febrilis**	—	—	LLL	—	—	24. iv. 16	27 ♂ 35 ♀	—
5	*Bibio hortulanus**	—	—	LLLL	—	—	5. iii. 17	♂s ♀s	29 and 30. v. 17
6	*Bibio Johannis**	—	LL	—	—	—	29. i. 16	1 ♂ 1 ♀	—
7	*Psychodidae**	—	L (?)	—	—	—	—. i. 17	—	6. iv.–1. v. 17
8	*Tipula* sp. 1*	L	—	—	—	—	12. ii. 16	—	—
9	,, sp. 2*	—	LL	—	—	—	28. ii. 17	—	1. v. 17
10	*Rhyphus fenestralis**	—	LLL	—	—	—	7. x. 16	—	1–28. v. 17
11	*Sargus cuprarius**	—	LLL	—	—	—	29. ii.; 3. iii. 16	♂s ♀s	June and July 16
12	*Chloromyia formosa**	L	—	—	—	—	March	1 ♀	—
13	*Microchrysa polita**	—	LL	—	—	—	3. iii. 16	3 ♂ 1 ♀	—
14	*Ascia podagrica**	LL	—	—	—	—	2. iii. 17	3 ♂ 1 ♀	28 & 30. iv. & 5. v .17
	,, ,,	—	LL	—	—	—	29. ii. 16	3 ♂ 1 ♀	—
15	*Eristalis tenax**	L	—	—	—	—	12. ii. 16	—	July 1916
16	,, *arbustorum**	—	L	—	—	—	1. i.; 26. ii.; 2. iii. 17	1 ♀	18. iv. 17
17	*Syritta pipiens**	—	LLL	—	—	—	29. ii. to 22. iii. 16	♂s ♀s	13–18. v. 17
	,, ,,	—	LL	—	—	—	22. ix. 16; —. i.; 23. iv. 17	3 ♂ 1 ♀	—
18	*Tachina* sp.*	—	—	—	—	—	21. viii. 16	1 ♀	14. v. 17‡
19	*Stomoxys calcitrans*	—	—	—	LLPP	—	7. ii. 16	1 ♀	—
20	*Graphomyia* sp.*	—	L	—	—	—	24. iii. 16	1 ♀	—
21	*Crytoneura caesia**	—	—	—	L	—	7. iii. 17	1 ♀	7. v. 17
22	*Morellia hortorum**	—	PP	—	—	—	—. ix. 17	15 ♂ 5 ♀	7–13. v. 17§

23	*Calliphora erythrocephala*	—	—	—	—	—	25. x. 16	♂ ♀	Jan. 1917‖
	,, ,,	—	—	—	—	—	1. i. 17	♂ ♀	9. ii. 17¶
	,, ,,	—	PP	—	—	—	—. i. 17	1 ♂	4. v. 17
24	*Hydrotaea dentipes*	—	LLPP	LLPP	LLLPPP	—	1. ii.; 13–23. iii.; 20. iv. 16	40 ♂ 28 ♀	—
	,, ,,	—	—	—	LLL	—	—. ii. to 7. iii. 17	6 ♂ 3 ♀	30. iv. & 2. v. 17
25	*Anthomyia radicum*	—	P	—	—	—	1. ii. 16	1 ♂	—
26	,, sp. 1*	P	—	—	—	—	2. iii. 17	1 ♂	30. iv. 17
27	,, sp. 2*	—	PP	—	—	—	—. ix. 16	—	9. v. 17
28	*Chortophila cinerella**	—	PPP	—	—	—	7. x. 16	♂ ♀	28. iii. & 16. v. 17
29	*Pegomyia socia**	—	P	—	—	—	22. ii. 17	—	30. iv. 17
30	*Fannia manicata*	—	—	—	—	LL	6. iv. 17	♂s ♀s	May 17
31	,, *scalaris*	LLL	—	—	LLL	—	—. 29. ii.; 13–22. iii. 16	125 ♂ 73 ♀ (26?)**	—
	,, ,,	—	—	—	LL	LLPP	6. iv.; 27. iii. 17	♂s ♀s	6. iv. 17
32	,, *canicularis*	—	—	—	LL	LLPP	6. iv. 17	♂s	6. v. 17
33	*Scatophaga stercoraria*	—	PP	—	—	—	29. i. 16	3 ♂s	4. v. 17††
34	*Tephrochlamys rufiventris**	—	—	PP	—	LLPP	22. iii. 16; 22. iii. 17	—	9–16. v. 17
35	*Lonchaea vaginalis**	—	PPLL	—	L	—	20. iv. 16	1 ♂ 3 ♀	7. 14 & 21. v. 17
							7. iii.; 28. iv. 17	1 ♀; ♀s ♂s	
36	*Sepsis* sp.*	—	PP	—	—	—	—. ix. 17	—	7–9. v. 17
37	*Borborus equinus**	aaaPP	aaaaPPPP	—	—	—	—	—	Throughout winter
38	*Limosina sylvatica**	—	LL	—	—	—	—. i. 17; 19. ii. 16	1 ♂	—
	,, ,,	—	—	P	—	—	22. x.; 23. iii. 17	2 ♂ 3	17. iv. 17
39	*Stenopteryx hirundinis**	—	—	—	—	—	25. x. 16	♂ ♀	12 & 14. v. 17‡‡

a, P or L denote the stage in which the insect was found, a = adult, P = pupa, L = larva. aa = a few adults, aaa = many adults, aaaa = very many adults.

† No sign of *Scatopse* larvae was seen when manure was first collected but they appeared later in the closed jam jar in the laboratory; it is possible that either eggs or very small larvae were in the manure provided for *T. rufiventris* but were not noticed.

‡ From cocoons of small Ermine moth (*Hyponomeuta* sp.). § (All females on 13. v. 17.)

‖ From Martins' nests. ¶ From soil in corner of slaughter-house yard. PPLL.

** 26 *F. scalaris* were destroyed and so it was impossible to determine their sex.

†† From human excrement, which had been exposed during autumn 1916. PPP.

‡‡ Reared separately by Mr N. D. F. Pearce and self from pupae found in Martins' nests.

Consequently the futility of calculations based on the number of larvae or of pupae per pound of manure is apparent.

6. In the majority of manure heaps, examined during the winter, there was no evidence that the larvae preferred any particular part of the heap; but in a few cases they seemed to show a slight preference for those portions which received least light.

7. On February 19th and 29th and on March 4th, 1916, several Chelifers—*Chernes nodosus*—were found in dryish cow manure, at a spot in the heap which registered a temperature of 35° C. On March 4th some were carrying their egg-masses. Large numbers of *Gamasid* mites were also found in the same heap.

8. The following parasites were bred—from two pupae of *Hydrotaea dentipes*, found in cow manure, 2 *Ichneumons* (*Atractodes tenebricosus*, Grav. ♀, *A. exilis*, Hal. ♂); from a third pupa of *H. dentipes* a *Figitid*; from pupa of *Lonchaea vaginalis* or of *Anthomyia radicum* an *Ichneumon*; from 2 *Calliphora*-like pupae, found in an ash midden, 1 and 2 *Figitids* respectively; and from 3 pupae of *Eristalis tenax* 72 (10 ♀ and 62 ♂), 24 and 8 (5 ♂ and 3 ♀) *Proctotrypids*—*Diapria conica*, Fabre, were extracted dead (see Kieffer and Marshall, 1907, pp. 948–950).

Observations on the Distribution of Flies during the Summer and Autumn 1916.

From July to September a certain number of places were visited to ascertain whether the House-fly and other species were present, and whether distance from a town or other houses affected the species or numbers of *Musca domestica*.

In some cases old fly papers and contents of traps were collected and fresh fly papers hung up; in two instances traps were set and baited; and in all cases flies present were caught and examined.

For this purpose the places examined were divided into five groups:

(*A*) = Places in town (Cambridge).

(*B*) = Places, other than houses, outside town near buildings.

(*C*) = Places, other than houses, outside town far from buildings.

(*D*) = Houses far from buildings.

(*E*) = Houses near buildings.

(*A*) During the summer the majority of *M. domestica* were found in the neighbourhood of horse manure, and were found to linger longest in artificially heated buildings, *e.g.* a bakery. The latest date on which a house-fly was found in the former situation was December 2nd, on which

day one was found, and in the latter situation, resting high up on the wall of a top-floor room and only moving on compulsion, December 9th.

(*B*) *M. domestica* was only found near horse manure except on two occasions—once in large numbers on moist brewer's grains in the troughs in a cow-house; and once a single female on rotting vegetation near a house on July 22nd. The majority of Diptera were *Stomoxys calcitrans*, *Scatophaga*, *Borborus equinus* and *Limosina* sp.

On one farm it was very noticeable that when the cows were brought in for milking in the afternoon they became very restless for the first five minutes after entering their stalls, kicking and lashing their tails. During this period no *Stomoxys* were to be seen in the sun on the cow-house wall outside; but afterwards, when the cows had become quiet, this wall was covered with *Stomoxys* freshly filled with blood.

(*C*) *M. domestica* was not found. The majority of Diptera found were *Scatophaga* sp., *Sarcophaga* sp., *Stomoxys* sp., *Borboridae* and *Anthomyidae*.

(*D*) Most of these houses were examined too late in the season to judge of fly prevalence in summer. One, examined on October 12th, had *Fannia canicularis* only. Traps set up in two derelict houses in Southery Fen, where 30,000 acres had been under water for 9 months—June 3rd, 1915 to September 1915, and again from May 1916 to December 1916—attracted 2 female *M. domestica*, 1 female *Stomoxys calcitrans*, 1 male *P. rudis* and 4 *C. erythrocephala*. The last mentioned laid eggs on the baits—human faeces, plums, milk and sheep's noses, which were placed together in the same receptacle.

Both houses were ¼ mile from the next derelict building; and one was ½ and the other 2 miles from land. Two of the four *C. erythrocephala* caught were found at the former house, the remainder at the latter.

During the interval between the two floods search of the fields for insect life resulted in finding only the larvae of *Chironomomus*, one *Syrphid* larva and a boring beetle grub—the latter being found in a post above water level.

Earthworms were only found in straw stacks, where leech cocoons were also found during the second flood.

During the second flood large numbers of Capsids were seen on isolated clumps of Willows surrounded by water.

(*E*) Large numbers of *M. domestica* were seen in farm houses during the summer, and many were caught in them and in a few cottages in October. On November 9th and 23rd a few were seen in the kitchen of a farm house.

A clean cottage had no flies, whilst one, to which it was attached and which was less clean, had many *M. domestica.* No flies were found on November 29th in a cottage, in which there had been large numbers on the 6th.

M. domestica was, therefore, found in largest numbers in town and in the country in the neighbourhood of horse manure—in the latter case in and about farmyards and buildings and in warm kitchens of farm houses. But the numbers were found to decrease markedly when isolated houses in the country or manure heaps far from buildings were examined. In farmyards and manure heaps far from buildings in the country such genera as *Stomoxys*, *Scatophaga*, *Borborus* and *Limosina* predominated.

M. domestica was observed alive in town as late as December 2nd outside, and December 9th inside: in the country as late as December 4th both outside and inside in 1916. In 1917 a house-fly was caught in a house in a village in Cambridgeshire during the last week in December, so that it is probable that those seen in that month in 1916 lingered sometime longer, but the places were not revisited in that year.

Observations on a Horse manure heap in Summer.

Observations were made on the temperature generated in a horse manure heap: the number of flies emerging therefrom: its attraction for adult flies and power to nourish their larvae after fermentation: and on a preliminary trial of the effect of an application of creosote oil mixture (see Forman and Graham-Smith (1917), pp. 123–199).

From July 31st to October 21st, 1916, three separate experiments—*A*, *B*, *C*—were carried out; each dealing with about 6 cwt. of horse manure, which was one day's accumulation from a stable in Cambridge.

The manure was, in each case, made into a heap in a specially prepared enclosure: and daily observations were made on temperatures recorded at certain points in the heap, on the number of flies emerging from it and the time of their emergence.

Experiment *A* dealt with 6 cwt. 1 qr. manure, and lasted from July 31st to August 14th. The manure was thrown into a loose heap. Experiment *B* dealt with 6 cwt. of manure which was tightly packed, and lasted from August 31st to October 29th. Experiment *C* dealt with 5 cwt. 1 qr. manure, which was loosely thrown up as in *A* but treated "incrementally[1]" with 1225 c.c. of creosote oil mixture. It lasted from September 18th to October 21st.

[1] This term implies that the manure was spread out in a thin layer upon the ground and the fluid then sprayed evenly over its surface.

Method.

A wooden enclosure was made 6′ 6″ × 7′, three sides of which were formed by a tray 4″ wide (Fig. 1 *LMQJ* and Fig. 2 *MKRP*), having an outer wall extending 1′ above and 6″ below ground. This tray was filled with sawdust and protected from wet by a sloping roof (Fig. 2 *MN*). The walls were sunk below the ground level to prevent the larvae from migrating, and the sawdust was to persuade them to pupate within the enclosure. The fourth side was closed by a hollow wall 3′ 6″ high, the inner side of which was sunk 6″ below the ground (Fig. 1 *DLIJ*). The 4″ between the back and front boards (Fig. 1 *GHDI*) was filled with

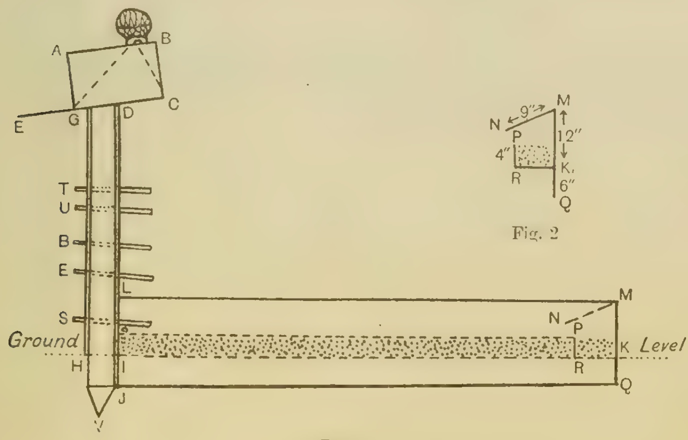

Fig. 2

Fig. 1.

Dotted Area = Sawdust.

sawdust to act as a non-conductor; and the wall was pierced in a centre line by five ½″ holes at points 3″, 6″, 12″, and 18″ from a point 2′ 6″ from the ground, which was to be the top of the heap.

The manure was placed against this wall so that its upper surface reached this point 2′ 6″ from the ground. Tin tubes, closed at one end, were then inserted through these holes so that they projected 6·5″ into the heap. Temperatures were taken by inserting a centigrade thermometer into each tube. The thermometer used could be read without removing it from the tube; and was provided with a piece of rubber tubing which fitted on to the open end of the tin tube into which it was inserted, thus preventing warm air from escaping from the tube. When

the tubes were empty their outer ends were closed with rubber corks. In this way temperatures were taken daily at several points in the heap

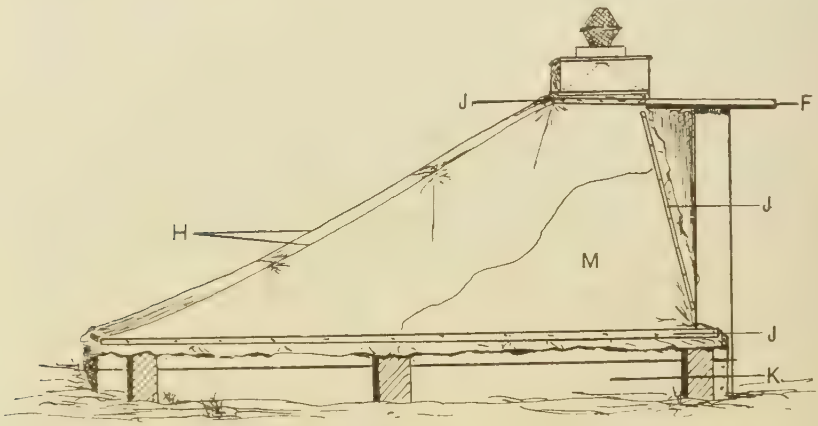

Fig. 3.

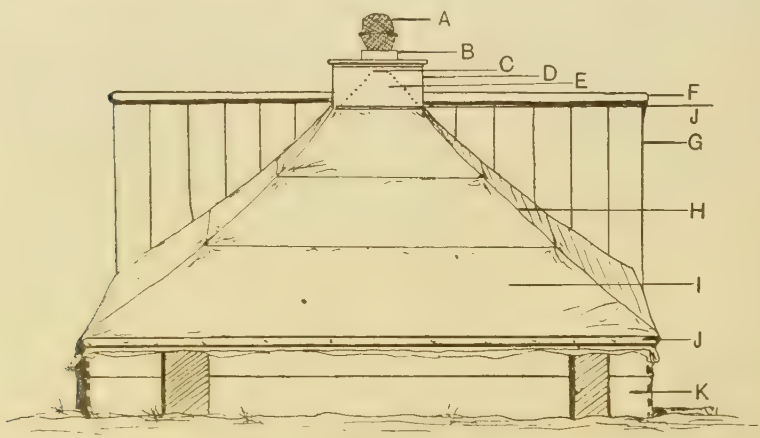

Fig. 4.

Figs. 3, 4. *A*, balloon trap: *B*, wooden square with hole in centre to hold trap: *C*, lid of tobacco tin (with hole in centre) to catch muslin: *D*, square wooden box: *E*, muslin, soaked in black ink caught under tobacco tin and at sides of box: *F*, roof to shelter thermometers: *G*, wooden wall against which manure was placed: *H*, support wires: *I*, muslin: *J*, lath, holding muslin: *K*, outside of tray containing sawdust (Figs. 1, 2): *M*, manure.

without disturbing the manure. The thermometer was left in each tube for 10 mins.—the temperature becoming constant in that time.

In order to collect the flies which emerged, the heap was enclosed by means of muslin supported on wires (see Figs. 3 and 4) so arranged that all sides sloped to an inverted box. Inside this box a muslin funnel was constructed by nailing muslin to the sides and pulling it down to the centre by catching it between the bottom of the box and the lid of a round tobacco tin. Thus all the flies were guided to a small skylight at the end of the muslin funnel, over which was set a balloon trap.

In order to find out whether manure, which had almost ceased to ferment still had attraction for adult flies or nourishment for their larvae, a sample of horse manure was taken from a heap when it had ceased to ferment. Part of this was placed in a large biscuit tin, provided with a very fine gauze-covered hole in the lid to admit air, into which 66 half-grown larvae of *M. domestica* were placed. On September 28th 8 pupae were found, from which only one *M. domestica* emerged. From another portion of the sample a water extract was made by soaking it in a bucket of water for 36 hours, stirring at intervals, straining the liquid through coarse muslin and evaporating it over a waterbath to the consistency of rather liquid porridge. A watch glass containing some of this extract was then placed in a cage with 36 *M. domestica* (9 ♂ and 25 ♀) which had been fasted for a week. They took practically no notice of it and were all dead in about 10 days.

Summary of observations.

The results of the three experiments may be summarised thus:

1. In a loosely formed horse manure heap the maximum temperature is reached during the first 3 days from the time the heap is formed, provided that the manure is not more than 24 hours old when so treated; and subsequently a fairly rapid and steady fall takes place throughout the heap. This may be influenced, to some extent, by external temperature (Chart I).

2. In a tightly packed heap the temperature registered by the superficial portions resembles the temperatures recorded throughout a loosely packed heap. On the other hand, the deeper portions tend to remain at high temperature for a long time and then to fall suddenly (Chart II).

3. A comparatively lower mean temperature and a quicker fall was registered in experiment C than in either experiments A or B. In the former case they were due chiefly to a corresponding decrease in the temperature of the air, but in the latter case chiefly to the fact that the mean temperature of the manure in experiment B was kept relatively

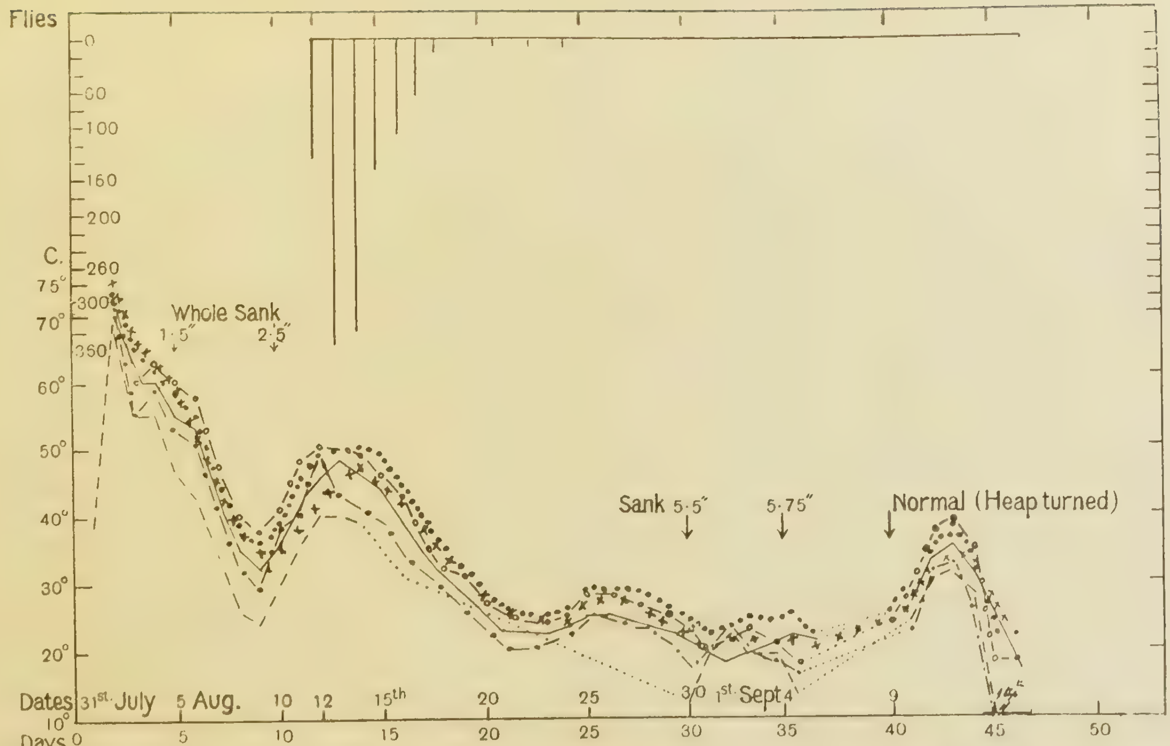

CHART I. *Experiment A.* 6 cwt. 1 qr. of horse manure made in town stables on July 31–Aug. 1, 1916: *loosely* thrown into a heap on Aug. 1. Temperature taken first on Aug. 2: heap exposed till Aug. 10, then enclosed with muslin. Flies came out between Aug. 12 and 23—total 1142, of which 1132, *i.e.* 99 per cent. were *Musca domestica* (48 per cent. ♂, 52 per cent. ♀). Heap sank a little as marked.

Curves of average daily temperature at points 6″ into back of heap.

———————	Mean of all five curves				
— — — — —	Hole 1 at commencement	3″ from top of heap			
—.—.—.—.—	,, 2	,, ,,	6″	,,	,,
—o—o—o—o—	,, 3	,, ,,	12″	,,	,,
....	,, 4	,, ,,	18″	,,	,,
× × × × × × ×	,, 5	,, ,,	24″	,,	,,

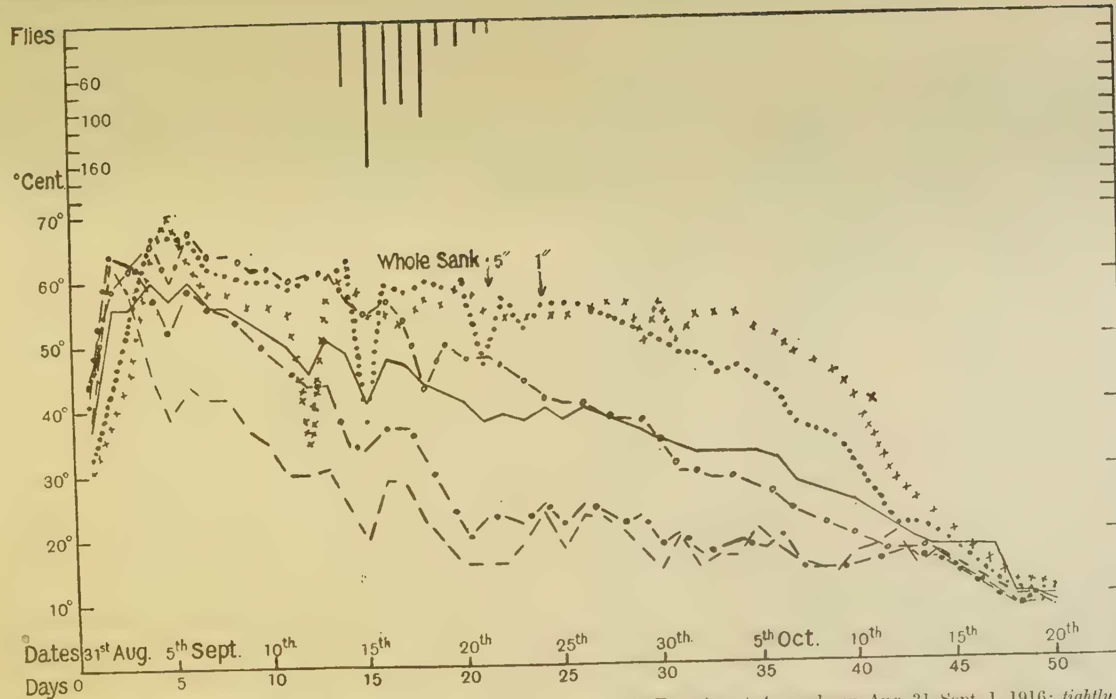

CHART II. *Experiment B.* 6 cwt. horse manure from same source as in Experiment *A*: made on Aug. 31–Sept. 1, 1916: *tightly* packed into a heap on Sept. 1. Temperature taking started Sept. 1, finished Oct. 20. Heap enclosed Sept. 13. From Sept. 14 to 29 678 flies caught—673 were *M. domestica*, 99 per cent. (45 per cent. ♂, 55 per cent. ♀). Heap sank a little as marked.

Curves of average daily temperatures at point 6″ into back of heap and curve of mean of all five.

————————	Mean of all five curves				
— — — — —	Hole 1 at commencement	3″ from top of heap			
—.—.—.—.—	,, 2 ,, ,,	6″	,,	,,	
—o—o—o—o—	,, 3 ,, ,,	12″	,,	,,	
....	,, 4 ,, ,,	18″	,,	,,	
× × × × × × ×	,, 5 ,, ,,	24″	,	,,	

high by packing and possibly, to a much less extent, to the mean of *C* having been reduced by the effect of creosote oil mixture on fermentation (Chart III).

4. The flies began to emerge about the 12th day, the majority appeared in 5 days; the period of emergence lasted about a fortnight. 2083 of the flies, which emerged in all three experiments, were caught in the trap and counted; 99·5 per cent. of these were *Musca domestica* (1141 in *A*, 680 in *B*, and 262 in *C*).

5. Male *M. domestica*, as a whole, started emerging rather sooner than the females.

6. Creosote oil mixture when sprayed incrementally at the rate of a gallon to the ton of horse manure did not entirely inhibit developement of flies. But as these experiments were not carried out simultaneously, it was not possible to judge of its precise effect from this experiment (see pp. 78–80).

7. Horse manure, which has almost finished fermenting and is cold, has probably no attraction for adult house-flies, and very little nourishment for their larvae, though larvae of other species have been found therein throughout the winter and flies, in some cases, successfully reared.

(For Temperature Curves see Charts I, II, III.)

A further Experiment to Discover the Amount of Creosote Oil Mixture necessary to spray upon Manure to Prevent Developement of Flies, and the best method of Application.

On February 26th, 1916, one day's production of horse manure, procured from the same source as for the previous experiment, was divided into five lots of 1 cwt. each. Each lot was then spread out separately in a stable yard in layers about a foot deep, to allow of a possible re-infection by flies. After 4 days exposure each plot was wetted with half a bucket of water to make it about as damp as when first spread, packed into tubs (Fig. 5) and treated as below:

No. of Tub	Treatment
1 (control)	Packed down tightly
2	Packed down tightly and sprayed on surface with fine spray with 218 c.c. Creosote Oil Mixture = 1 gallon to 1 ton
3	Packed down tightly and sprayed on surface with fine spray with 873 c.c. Creosote Oil Mixture = 4 gallons to 1 ton
4	Packed down tightly and sprayed "incrementally" with fine spray with 218 c.c. Creosote Oil Mixture = 1 gallon to 1 ton
5	Packed down tightly and sprayed "incrementally" with fine spray with 873 c.c. Creosote Oil Mixture = 4 gallons to 1 ton

On October 2nd several larvae of *M. domestica* were seen on the inside of the control tub, escaping from the excess of heat. The temperature about 6 inches below the surface was, at that time, about 67° C. No life was apparent in the other tubs. On October 3rd many very small flies and a few *Psychodidae* were seen inside the muslin covering the control, and very many very small larvae were seen energetically writhing at the ends of protruding straws, as if trying to escape from the heat below; a dead beetle was found on No. 4 and a few live beetles on the remainder. On October 11th 1 cwt. horse manure, procured from the

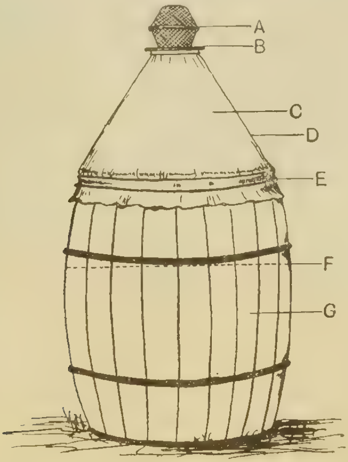

Fig. 5. *A*, balloon trap: *B*, wooden square with hole in centre supported by four wires fastened into hoop inside tub: *C*, muslin: *D*, supporting wire: *E*, tape to hold muslin: *F*, surface of manure (1 cwt.): *G*, wooden tub.

same source and treated in a similar way to the first 5 lots, was placed in tub No. 6 and sprayed "incrementally" with a solution of borax (1 oz. borax, 1 qt water: 1 cu. ft manure).

On the first 5 days tubs 2, 3 and 4 gave higher temperatures than the control; but No. 5, which had been sprayed incrementally at the rate of 4 gallons to 1 ton, showed a lower temperature on the first three days and a higher on the fourth and fifth. This seems to suggest that Creosote Oil Mixture, when applied to the surface, tends to retain the heat generated below, so that the heap takes longer to cool; but that if applied incrementally in sufficient quantity, fermentation is delayed and the temperature rises more slowly (Chart IV).

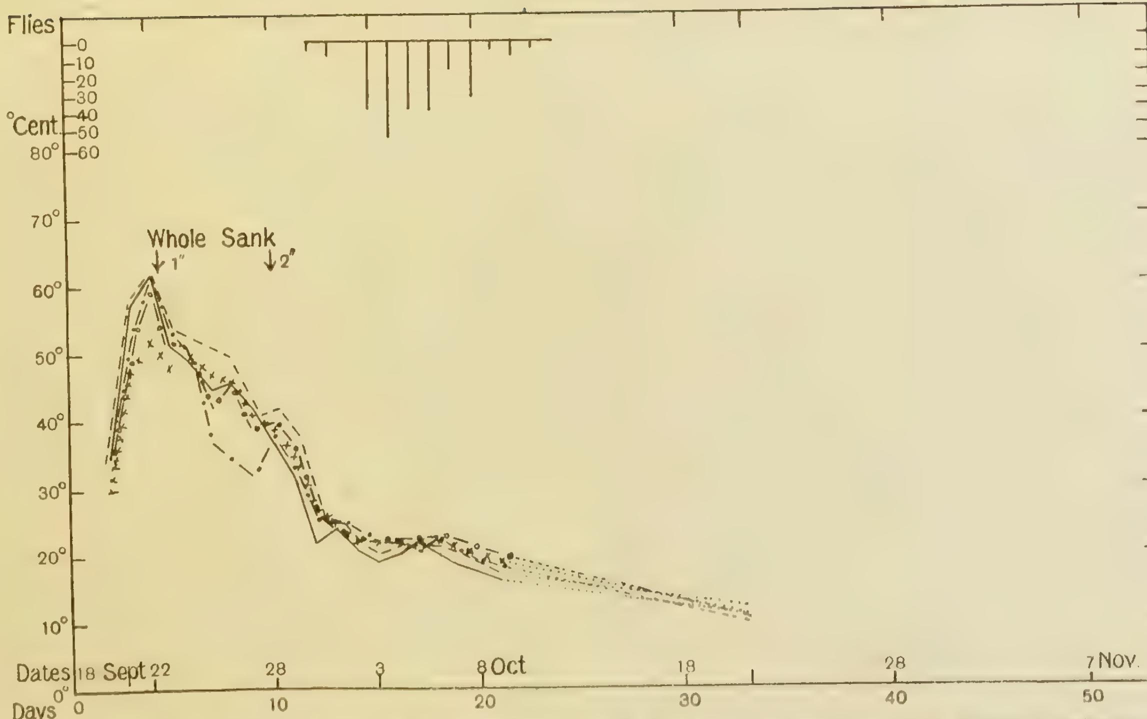

CHART III. *Experiment C.* 5 cwt. 1 qr. of horse manure made in town stables on Sept. 18–19, 1916: brought to School of Agriculture on Sept. 19 and spread out to a depth of a few inches: sprayed by means of a hand syringe with 1200 c.c. of creosote oil mixture, *i.e.* 1 gallon to 1 ton. (50 c.c. were sprayed on surface on which heap was to rest: the manure was spread in quarters, each receiving 250 c.c., leaving 150 c.c. for surface of heap. An extra 25 c.c. was added because of wind.) 312 flies emerged between Sept. 30 and Oct. 14. Later heap sank a little as shown.

Curves of averages of daily temperatures at five points 6″ into back of heap and, at beginning of experiment 3″, 6″, 12″, 18″ and 24″ from top of heap.

————————	Mean of all five curves					
— — — — —	Hole 1	at commencement		3″	from top of heap	
—.—.—.—.—	,, 2	,,	,,	6″	,,	,,
—o—o—o—o—	,, 3	,,	,,	12″	,,	,,
....	,, 4	,,	,,	18″	,,	,,
×××××××	,, 5	,,	,,	24″	,,	,,

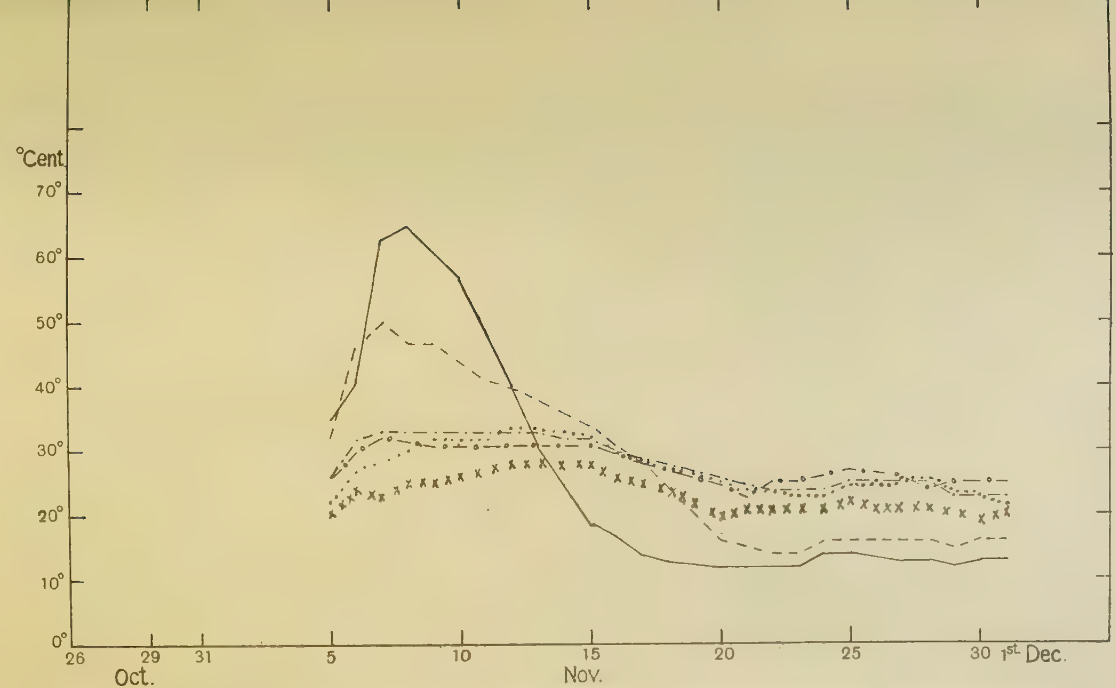

CHART IV. *Experiment. Curves showing the daily average temperature of the manure in each tub, as registered at points 3″, 6″ and 12″ from the surface.*

Treatment of manure in each tub.

1. Control, only pressed

"Incrementally" { 2. Sprayed with creosote oil mixture (2 galls to 1 ton)
3. ,, ,, ,, (4 galls to 1 ton)
4. ,, ,, ,, (8 galls to 1 ton) }

Surface { 5. Sprayed with borax (at 1 oz. to 1 qt of water to 1 cub. ft manure)
6. ,, ,, ,, ,, ,, ,, ,,
then sprayed with creosote oil mixture (1 gall. to 1 ton) }

————————	1	—.—.—.—.—	3	....	5
— — — — —	2	—o—o—o—o—	4	×××××××	6

A little life was evident in all five tubs on the first three days; but, whereas the control gave 81 *M. domestica* between October 10th and November 13th, No. 2 (surface 1) gave 1418, No. 3 (surface 4) 130, No. 4 (incrementally 1) 3, and No. 5 (incrementally 4) 2; whilst No. 6 (borax) gave 16.

The difference between the flies emerging from the control and from tub 2 may have been due to uneven sampling of the manure. Nevertheless the figures 2 and 3 contrast sufficiently with those of 81, 1418 and 130 to justify the following suppositions:

1. That the larvae are able to live between the great heat generated below by the manure and the larvicide sprayed on the surface only. (The temperature 1 in. below was 41° C. on the fifth day, *i.e.* when the manure was 19 days old.)

2. That the best method to apply Creosote Oil Mixture is "Incrementally."

3. That 1 gallon to the ton is sufficient; but a previous trial indicated that more is needed.

As the first experiment seemed to show incremental spraying to be more effective than surface treatment, it was decided to repeat the experiment with variations in quantities of solution, and to make a further trial of borax.

On October 30th, 1916, horse manure procured from the same source was thoroughly mixed to ensure even sampling, divided into six lots each of 1 cwt. 1 qt. and exposed as before. On November 20th they were treated as follows:

1. (Control) pressed into tub.
2. Sprayed incrementally with 436 c.c. Creosote Oil Mixture = 2 gallons to 1 ton and pressed into tub.
3. Sprayed incrementally with 872 c.c. Creosote Oil Mixture = 4 gallons to 1 ton and pressed into tub.
4. Sprayed incrementally with 1744 c.c. Creosote Oil Mixture = 8 gallons to 1 ton and pressed into tub.
5. Watered with can with 5 oz. Borax to 5 quarts water to 5 cu. ft manure.
6. Watered with can with 5 oz. Borax to 5 quarts water to 5 cu. ft manure, and later sprayed on the surface with 218 c.c. Creosote Oil Mixture, *i.e.* 1 gallon to 1 ton.

The treatment of No. 6 with borax was an attempt to drive the larvae up into the Creosote Oil Mixture.

Holes were drilled through the tubs at points 3 in., 6 in. and 12 in. from the surface, and tin tubes as used in a previous experiment (p. 69) were inserted to about 8 in. Temperatures were taken daily; and an average of the three holes calculated for each tub.

The untreated control showed a higher and quicker rise and a lower and more sudden fall than any of the treated tubs. A consideration of the other curves seems to indicate that treatment of manure with either Creosote Oil Mixture or with borax considerably delayed fermentation. So that such manure never gave temperature readings as high as the untreated and took longer to fall to the same level.

This experiment was started late in the season, when the manure possibly contained fewer eggs when collected and sufficient flies did not emerge from it to make the result decisive.

Though no flies emerged from tub 5 in this experiment, they did emerge from tub 6 of the first experiment, which was similarly treated. However, it may be noted that (1) the same quantity of very small flies emerged from the control and from No. 2 and about half that quantity from No. 3, but none from the remainder; (2) of the larger flies (*Scatophaga* and *M. domestica*) most (44) emerged from the control, whilst from each of Nos. 2 and 3, 3 specimens emerged; (3) there was no life in 4, 5, 6 (8 galls of Creosote Oil Mixture to 1 ton manure; borax; and borax plus 1 gall. Creosote Oil Mixture to 1 ton manure); (4) larvae were visible at any time in the control only; and no flies were caught over 4, 5 and 6, which would seem to show that the treatment of 4, 5 and 6 prohibited fly developement; (5) none of the larger flies emerged from these tubs in the spring of 1917.

The results of both these experiments may be summarised thus:

1. Surface sprayed manure gave higher temperatures than the unsprayed control, but though there was no sign of larval life on the surface at any time, fly developement was not prevented.

2. Larvae seem to be able to live just below the surface between the great heat below and the layer of Creosote Oil Mixture above. The temperature 1 inch below was, in one case, 41° C., which has been quoted as being the lethal temperature for larvae of *M. domestica* (Copeman, 1916).

3. Incremental treatment delays fermentation and consequently rise in temperature.

With the use of Creosote Oil Mixture in quantities up to 2 gallons to 1 ton of manure the temperature curve, though it does not rise so high, resembles that of the untreated manure; but larger quantities and borax, at the rate of 1 oz. borax to 1 quart of water to 1 cu. ft of manure altered the curve considerably. There was no rapid rise or fall as in the untreated heap—30° C. was hardly exceeded and 21° C. was the lowest temperature reached by manure so treated: whereas the untreated gave readings of 65° C. and 12° C.

No. 6 in experiment 2, treated with borax and creosote oil mixture gave still lower mean temperature.

4. Owing to possible uneven sampling of the manure in the first experiment and lateness of the season of the second, the minimum effective quantity of creosote oil mixture was not determined. But it should be noted that it seemed doubtful whether 1 gallon to 1 ton of manure would prove sufficient, whilst 4 gallons did prove effective.

5. Incremental treatment is superior to surface treatment.

6. Though the 6 tubs in experiment 2 were under observation until spring 1917 no *M. domestica* emerged from any of them after the winter.

Experiment to attempt to determine (1) the depth at which it is necessary to bury material infested with fly larvae, in different soils, to prevent the escape of the adult, (2) the effect of filling in the pits loosely or of ramming the soil; and (3) the distance through which the adult flies are able to climb when emerging from pupae in different soils, loosely or tightly packed.

In the *Military Manual of Elementary Hygiene*, 1912, two methods are given by which to dispose of excreta: (1) In the "Long and deep trench system" a trench 5 yards long × 16 inches wide × 3 feet deep is the allowance for 100 men; and "the contents of latrine trenches should be covered with a couple of inches of dry earth daily." (2) In the "Short and shallow system" the trench is made 1 foot deep and lasts 1 day, unless the contents is levelled down and the covering of earth is finely broken down, when it may be made to last longer." In paragraph 13, page 65, one is told that "so soon as the latrine-trenches have been filled in to within *six inches* of the ground level their use should be discontinued, earth thrown in, and turf or sods replaced."

As regards refuse it states that in bivouacs and camps of a temporary nature these receptacles (for disposal of refuse) may take the form of pits or holes, but where these are employed, the contents must be covered over with at least six inches of earth two or three times a day to prevent flies being attracted to them.

No instructions to vary the depth according to the nature of the soil are given. A couple of inches of dry earth thrown over the excrement would probably be insufficient to prevent the material becoming infested with flies' eggs: and six inches has been proved too little to prevent the escape of any flies developing (see Graham-Smith, 1916, p. 503).

Henri Fabre found that *Sarcophaga carnaria* could traverse a column of sand six inches high in 15 minutes; and in an experiment, in which he placed lots of 15 pupae of *Calliphora* at the bottom of large glass tubes, which were then filled with different quantities of sand, found that from those pupae covered with

6 cm. (2·34″)	of sand	14	out of 15 flies	reached	the	surface
12 cm. (4·68″)	,,	4	,,	,,	,,	,,
20 cm. (7·8″)	,,	2	,,	,,	,,	,,
60 cm. (23·4″)	,,	1	,,	,,	,,	,,

Hewitt (1915) found pupae of *M. domestica*, from which flies had successfully emerged, 24 inches below the ground in light soil.

Graham-Smith (1916) made the following observations on burial of infested material: (1) "On July 7th a large piece of sheep's lung containing some eggs and very small larvae was buried to a depth of 1½ feet, and the earth well packed down. The earth was further trodden down on July 9th. On July 12th the material was exhumed and numerous large, apparently healthy, larvae were found in it." (2) "On September 4th the bodies of six guinea-pigs were exposed. On the same day one of the carcasses with eggs in the mouth and on the hair was buried to a depth of one foot in an earthenware pipe sunk vertically in the ground. A little earth was placed above the carcass and packed down tightly, and the process repeated till the pipe was full. The top of the pipe was sealed with an earthenware saucer. The other carcasses containing larvae of various sizes were similarly buried on September 8th, 10th, 11th and 14th. On September 26th full fed larvae and pupae were noticed just below and on the surface of the earth in all the pipes. Blow-flies began to emerge simultaneously from all the pipes on October 17th, and large numbers were caught up to October 29th, and smaller numbers up to November 15th. These observations show that larvae emerge from eggs and thrive in buried carcasses, and that they are able to make their way to within a short distance of the surface where they pupate. And that the burial of a carcass does not prevent flies emerging from eggs already laid, but only limits the production of flies by preventing later batches of eggs from being deposited."

The greatest depth tested by Fabre and by Graham-Smith was 23·4 inches and 18 inches respectively.

During May 1917, a hole *ABCD* (Fig. 6) about 4 ft × 4 ft × 4 ft 6 in. deep with an approach trench *GCEF* was dug in light loam soil. The floor was covered with sand to a depth of two inches to enable the

concrete to bind. A two inch layer of concrete was spread over the sand and six roughened slates were sunk into it; and a land drainage pipe one foot long and six inches in diameter was then placed upright on each of the slates on to which it was carefully cemented. When the cement had set the hole was filled in with earth to the level of the top of the pipes. A layer of sand was then spread round the mouths of the pipes and a second lot of pipes was cemented on to the first lot with a fresh layer of cement, which was sloped up to the pipe to ensure covering the joint completely. This process was continued until there were four tiers of pipes, *i.e.* until there were six holes six inches in diameter and four feet deep, from which no larvae could escape save upwards. The pipes were then numbered as shown in the diagram.

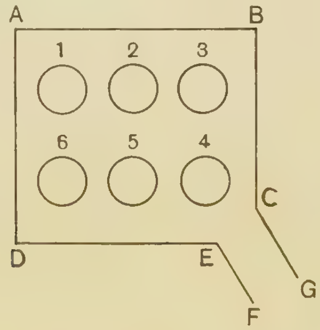

Fig. 6.

Many *C. erythrocephala* females were placed in a large muslin cage (Graham-Smith, 1914, Pl. XIX, fig. 3) with bodies of four guinea-pigs and of one large rabbit, which had been dead for several days, in order that they might thoroughly infest the carcasses with their eggs.

On May 2nd the body of a dead guinea-pig, which was swarming with larvae of *Calliphora*, was dropped into each of holes 1 and 2, which were then filled in with loam—the soil in 1 being loosely packed, but that in 2 tightly. On May 4th similarly infested remains were placed in 3, 4, 5 and 6; the first two being filled with sand and the last two with clay. Nos. 3 and 5 were loosely filled, but 4 and 6 tightly packed. The contents of 2, 4 and 6 were packed by filling them by degrees and ramming each lot of soil down with a long wooden pole.

A trap to catch any flies which might emerge was constructed over each pipe. A small piece of wood, with a half-inch hole in the middle, and to which muslin was tacked (Fig. 5), was supported on four wires,

which were pushed firmly into the soil round each pipe. The edges of the muslin were covered with soil, and a balloon trap was placed over the hole in the wooden top.

Flies emerged from all the pipes between July 1st and August 28th, 1917. A total of 2516 was caught and 9 species represented (see Table II).

Between December 29th, 1917, and January 2nd, 1918, the earth round the pipes was dug out, and sections were taken from each pipe at levels of 1, 8, 12, 18, 24, 30, 36, 42 and 48 inches, in the following way. The blade of a hack-saw, which had been marked at distances of 1 and 3 inches from one end, was pushed down the inside of a pipe until the inch mark was level with the top of the pipe. The soil was then carefully removed to that level; the blade being worked round to ensure not going too deep. The same procedure was used to obtain the 3 inch sections. The 8, 18, 30 and 42 inch levels were determined by careful measurements outside the pipes: a hole being made at the point with a cold chisel. The flat blade of a hack-saw was then thrust in horizontally, through the hole made, and the earth removed to that level. The 12, 24, 36 and 48 inch levels occurred at the cement joints, which were opened by means of a cold chisel. By thrusting in a piece of slate, it was then possible to remove the rest of the pipe and push from it the remaining section of soil.

Each section, as extracted from the pipe, was placed in a biscuit tin and marked; dried separately and passed through a 3 mm. sieve over white paper: pupae and remains of flies being extracted. The results are shown in Table III.

Owing to unavoidable absence from Cambridge the traps were not visited every day. On August 5th, after 7 days continuous rain, the muslin was found to have worn through round the rims of the pipes. The rents were repaired immediately, but this occurred between the 28th and 35th days after that on which the flies started to emerge; and the flies continued to appear in the traps up to the 59th day. A certain number of flies, therefore, may have escaped during the 7 days above mentioned, which may account for the discrepancy between the number of pupae found and flies caught in pipes 2 and 3.

A few pupae were noticed outside in the soil surrounding the pipes, which indicates that a certain number of larvae probably made their way completely through the 4 feet of soil and pupated outside.

The animal matter at the bottom of all the pipes except 2 and 6 was entirely consumed, only the bones being left. In 6 there remained a little dry skin and hair attached to some of the bones, but in 2 the greater

part of the animal still remained, forming a moist evil smelling mass of hair and rotten flesh. This would suggest that the larvae in pipe 2 died for some reason, which would account for the comparatively low numbers of pupae found and flies caught from it.

Owing to unavoidable delay (4 months) in opening the pipes, it is possible that a number of pupa cases and bodies of adult flies had rotted beyond recognition. Only five bodies of adult flies were found and they were much decayed. Three of these bodies were found in the loose clay, being between the 1 inch and 3 inch levels, and probably had died of exhaustion, whilst of the other two, one was found between the 30 and 36 inch levels, and the other between the 40 and 48 inch levels. The fifth was found in the rammed clay between 42 and 36 inch levels.

Some of the pupae which were found at the lower levels were ill-formed, but those in pipes 8, 2 and 3 were normal healthy ones. Only five were found to have been parasitised, though some of those which had disintegrated may have been so.

From this experiment two facts seem evident, namely, (1) flies are able to develope from larvae which have been buried as deep as 4 feet, and that it is, therefore, useless to bury larvae-infected material, even at that depth, to prevent fly developement; and (2) the majority of larvae (90 per cent. in this experiment) make their way up to within a foot of the surface of the soil before pupating.

TABLE II.

Showing numbers of flies which emerged between July 1st and August 28th, 1917, though their larvae had been buried within food material four feet below the surface of ground.

	Loam				Sand				Clay (dryish)			
	Loose 1		Rammed 2		Loose 3		Rammed 4		Loose 5		Rammed 6	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Calliphora erythrocephala	104	106	5	5	161	127	266	407	348	341	91	102
,, *vomitoria*	10	21	2	0	1	5	18	44	57	93	—	1
Lucilia caesar	1	1	—	—	—	—	4	7	19	29	5	4
,, *sericata*	1	2	—	—	—	—	6	7	38	26	6	—
Hydrotaea dentipes	—	—	—	—	31	4	—	—	—	—	—	—
Muscina stabulans	—	—	—	—	—	—	—	—	—	—	—	6
,, *pabulorum*	—	—	—	—	—	—	—	—	—	1	—	—
Small Anthomyidae	—	—	—	—	—	—	—	1	—	—	—	—
?	—	—	—	—	—	—	—	—	—	—	1	1
Totals (sexes)	116	130	7	5	193	136	294	466	462	490	103	114
Total flies	246		12		329		760		952		217	
	2516											

Table III.

Showing pupae and remains of flies found at different levels; and the per cent. of larvae which had climbed to within one foot of the surface before pupating. + = *remains of a fly.*

	Loam				Sand				Clay					
	1 Loose		2 Rammed		3 Loose		4 Rammed		5 Loose		6 Rammed		Section totals	
From surface														
to 1″ below	4	113	0	18	0	650	6	541	5	706	51	144	66	
1″– 3″	50	i.e.	0	i.e.	1	i.e.	445	i.e.	111	i.e.	13	i.e.	620	2171
3″– 8″	45	88	0	86	560	95	81	97	541	96	69	92	1295	i.e. 95 %
8″–12″	14	%	18	%	89	%	9	%	49	%	11	%	190	
12″–18″	5+		0		22		5		16		8		56	
18″–24″	0		0		3		6		10		2		21	
24″–30″	3		1		3		3		3		1		14	
30″–36″	2		1		4		0		+		0		7	
36″–42″	3		1		2		0		1		1		8	
42″–48″	1		0		3		3		+		0		8	
Total pupae	127		21		687		558		736		156		2285	

Summary of Investigations.

During the winters of 1915–1916 and 1916–1917 manure heaps and other likely places were searched for dipterous larvae and pupae. During the summer (1916 and 1917) observations were made on fly distribution in town and in country and various experiments were carried out to investigate the temperature conditions in loosely packed and tightly packed manure heaps, and the influence on those conditions and on fly developement of applications of creosote oil mixture and of borax in various quantities; to determine the amount of creosote oil mixture necessary to apply to prevent fly developement and the best way to apply it; to determine whether horse manure which had ceased to ferment was still attractive to adult flies or could provide nourishment for their larvae; to find out at what depth fly-larvae-infested material may safely be buried in different soils, loose or rammed, to ensure no flies emerging, and to what height the larvae would climb in those soils before pupating.

The results may be summarised thus:

1. Thirty-nine species of fly were bred from larvae or pupae found in natural situations during the winter. Of these 31 are additional to Dr Graham-Smith's (1916) list.

2. Pupae of *Musca domestica* were found but the adults were not reared; even though, in one experiment, lots of 1 cwt. of horse manure were kept under observation in barrels from autumn until the following spring.

3. The distribution of dipterous larvae in a manure heap in winter, at any rate in the Eastern Counties, is extremely local and, therefore, calculations based upon the number of larvae or pupae per pound of manure are futile.

4. Usually there seemed to be little preference shown by the larvae for any particular part of the heap, but in a few cases they seemed to select the portions which received least light.

5. During the summer and autumn *M. domestica* was found in largest numbers, in town and in the country, in the neighbourhood of horse manure—in the latter case in and about farmyards and buildings, and in the warm kitchens of farm-houses. But the numbers were found to decrease markedly when isolated houses in the country or manure heaps far from buildings were examined.

6. In farmyards and manure heaps near buildings in the country such genera as *Stomoxys*, *Scatophaga*, *Borborus* and *Limosina* predominated.

7. *M. domestica* was observed alive in town as late as December 2nd outside and December 9th inside; but one was caught in a house in Grantchester during the last week in December, 1917. Probably those seen in 1916 lingered sometime longer, but these places were not revisited in that year.

8. Flies were bred during summer from material used in summer investigations as follows: human faeces—*Hydrotaea*, *Scatophaga*, *Sarcophaga*, *Muscina stabulans* and small *Anthomyidae*; pig manure—*Mydaea*, *Scatophaga*, *Sarcophaga* and small *Anthomyidae*; cow manure—*Morellia hortoum*, *Eristalis*, *Scatophaga*, *Limosina*, *Psycodidae* and *Borboridae*; horse manure—*Stomoxys*, *Borboridae*, *Limosina*, *Psychodidae* and small *Anthomyidae*; small carcasses—*Hydrotaea* and *Calliphora*.

For flies bred from larvae and pupae found in various manures during winter see Table I.

9. Horse manure, which has ceased to ferment and is cold, has no attraction for the adults of *M. domestica* nor nourishment for its larvae, though it still attracts small species such as *Limosina*, *Psychodidae* and *Borboridae*; and though larvae of certain species of fly have been found in it throughout winter.

10. 99·5 per cent. of the flies bred from horse manure, made in a town stable during 24 hours in the autumn, were *M. domestica*, which emerged during 14 days, commencing on the 12th day.

11. Male *M. domestica*, as a whole, started emerging sooner than the females.

12. The temperature in a loosely packed heap of horse manure rises

higher and falls sooner and lower than that of a tightly packed one. The maximum temperature in a loosely packed heap is reached about the third day.

13. The deeper portions of a tightly packed heap remain at a higher temperature for a longer time and then fall suddenly.

14. Creosote oil mixture should be sprayed "*Incrementally*" and not merely on the surface.

15. It is doubtful whether 1 gallon to the ton of manure is sufficient to prevent flies from emerging. Four gallons proved enough, but it was not possible to carry out sufficient experiments to determine the precise minimum quantity necessary to prohibit developement of flies.

16. Incremental applications of creosote oil mixture and of borax lower the general temperature of the heap. The temperature never rises as high and takes much longer to fall as low. This is probably due to fermentation being delayed.

17. If a heap is sprayed on the surface with creosote oil mixture, *M. domestica* larvae are able to live between the sprayed surface and the intense heat below. The temperature 1 inch below the surface of the manure, when that of the heap is near its maximum, is about 41° C.—a temperature of which Howlett wrote "It is improbable that they (larvae) could live long at anything over 41° C. (about 106° F.)"—see Copeman (1916).

18. It is useless to bury larvae-infested material at a depth of 4 feet in clay, loam or sand, whether loose or rammed down, as the majority of flies will emerge.

19. About 90 per cent. of the larvae, so buried, climbed to within 1 foot of the surface before pupating.

20. The following parasites were bred—from two pupae of *Hydrotaea dentipes* two *Ichneumons* (*Atrodectes tenebricosus* Grav. ♀, *A. exilis* Hal. ♂), from a third pupa of *H. dentipes* a *Figitid*; from pupa of *Lonchaea vaginalis* or *R. radicum* an *Ichneumon*; from two Calliphora-like pupae one and two *Figitids* respectively; and from three pupae of *Eristalis tenax* 72 (10 ♀, 62 ♂), 24 and 8 (5 ♂, 3 ♀), *Proctotrypids*—*Diapria conica*, Fabre, were extracted dead.

REFERENCES.

BISHOPP, F. C., DOVE, W. E. and PARMAN, D. C. (1915). Notes on certain points of economic importance in the biology of the House-fly. *Journ. Econom. Entomol.* VIII, pp. 54–71.

COPEMAN, S. M. (1913). Hibernation of House-Flies. *Reports to the Local Government Board on Public Health and Medical Subjects*, New Series, No. 85, pp. 14–19.

—— (1916). Prevention of Fly-Breeding in Horse Manure. *The Lancet*, June 10th, 1916.

Copeman, S. M. and Austen, E. E. (1914). Do House-flies hibernate? *Reports to the Local Government Board on Public Health and Medical Subjects,* New Series, No. 102, pp. 6–26.

Dove, W. E. (1916). Some notes concerning over wintering of the House-Fly, *Musca domestica,* at Dallas, Texas. *Journ. Econom. Entomol.* IX, pp. 528–539.

Fabre, J. H. *Souvenirs Entomologiques,* 19e Serie, Delgrave, Paris.

Forman, F. W. and Graham-Smith, G. S. (1917). Investigations on Prevention of Nuisances arising from Flies and Putrefaction. *Journ. Hygiene,* XVI, 2, pp. 119–226.

Graham-Smith, G. S. (1914). *Flies in Relation to Disease. Non-Blood-Sucking Flies.* Cambridge University Press.

—— (1916). Observations on the Habits and Parasites of Common Flies. *Parasitology,* VIII, pp. 440–544.

Griffith, A. (1908). Life-History of House-flies. *Pub. Health,* XXI, pp. 122–127.

Hermes, W. B. (1911). The house-fly in its relation to public Health. *University of California publications, Bull.* Nov. 215, pp. 513–548.

Hewitt, C. G. (1910). The House-Fly. *A Study of its Structure, Development, Bionomics and Economy.* Biological Series, No. 1. Manchester University Press.

—— (1912). *Fannia* (*Homalomyia*) *canicularis* Linn. and *F. scalaris* Fab. An account of the Bionomics of the Larvae and their relation to Myiasis of the intestinal and Urinary Tracts. *Parasitology,* v, pp. 161–174.

—— (1914). Further Observations on the Breeding Habits and Control of the House-fly, *M. domestica. Journ. Econom. Entomol.* VIII, 3.

—— (1914). Observations on the feeding Habits of the Stable Fly. *Transactions of the R. S. Canada,* Series III, VII.

—— (1915). Pupation and Over wintering of the House-fly. *Canadian Ent.* XLVII, No. 3, pp. 73–78.

Howard, L. O. (1911). House Flies. *U.S. Dep. Agri. Farmers Bull.* No. 459.

—— (1912). *The House Fly, Disease Carrier. An Account of its dangerous activities and of the means of destroying it.* John Murray, London.

Kieffer, l'Abbe J. J. et le Rev. T. A. Marshall (1907). Les Proctotrypidae, Pt 2, in André, *Species des Hyménoptères,* Tome X.

Kisliuk, M. (1917). Some Winter Observations of Muscid Flies. *Ohio Journ. Sci. Columbus,* XVII, No. 8 (*Rev. App. Ent.* v, Ser. B, Sept. 1917).

McDonnell, R. P. and Eastwood, T. (1917). A Note on the Mode of existence of Flies during winter. *Journ. R.A.M.C. London,* XXIX, 1.

Newstead, R. (1907). *Preliminary Report on the Habits, Life Cycle and Breeding places of the Common House-Fly* (*Musca domestica, Linn.*), *as observed in the city of Liverpool, with suggestions as to the best means of checking its increase.* C. Tinling and Co., Liverpool.

—— (1909). *Second Interim Report on the House-Fly, as observed in the City of Liverpool.* C. Tinling and Co., Liverpool.

Portchinsky, J. A. (1913). Review in *Review of Applied Entomology,* 1, B, pp. 149–152.

Skiner, H. (1913). How does the House-Fly pass the Winter? *Entom. News, Philadelphia,* XXIV, pp. 303–304.

Williston, S. W. (1908). *Manual of North American Diptera.* James F. Hathaway, New Haven.